Assessment of a Molecular Marker Method to Determine the Pyrogenic Carbon Component in Charcoals and Soils

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Zusammenfassung

Holzkohle und Russ sind das Ergebnis unvollständiger Verbrennung, die bei Vegetationsbränden oder der Verbrennung fossiler Brennstoffe entstehen. Spuren dieser Rückstände können in praktisch allen Umweltkompartimenten nachgewiesen werden. Der in den Verbrennungsrückständen enthaltene pyrogene Kohlenstoff (PyC) stellt einen kleinen, aber bedeutsamen Anteil des globalen Kohlenstoffkreislaufs dar. PyC liegt hauptsächlich in Form aromatischer Moleküle vor, welche durch Hitzeeinwirkung aus organischer Substanz oder Biomasse hervorgegangen sind. Generell steigt mit zunehmendem Grad der Hitzeeinwirkung deren Kondensationsgrad und damit die Stabilität der Verbrennungsrückstände an. Aufgrund der Tatsache, dass PyC keine definierte Molekülstruktur aufweist, sondern eine breite Gruppe verschiedenster, durch Feuereinwirkung veränderter Substanzen umfasst, sind neben der Quantifizierung von PyC Informationen über deren molekulare Eigenschaften für eine vollständige Beschreibung unerlässlich. Um den globalen Kreislauf von PyC zu erfassen wird eine genaue Schätzung der Quellen, Flüsse und Senken von PyC in der Umwelt benötigt. Dazu ist eine Messmethode erforderlich, welche in der Lage ist, PyC zuverlässig in allen Umweltkompartimenten zu erfassen und zu beschreiben. In der vorliegenden Arbeit wird eine Methode eingesetzt, die sieben Benzolpolycarbonsäuren (BPCA) als spezifische Markermoleküle für PyC verwendet. Die BPCA-Methode ist eine vielversprechende Möglichkeit zur Charakterisierung von Verbrennungsrückständen in der Umwelt.

Im Rahmen dieser Arbeit wurde die BPCA-Methode systematisch getestet und zwei Nachweismethoden für BPCA miteinander verglichen. Kastanienholz (*Castanea sativa* Mill.) und Reisstroh (*Oryza sativa* L.) wurden unter Laborbedingungen bei Temperaturen zwischen 200 °C und 1000 °C pyrolysiert. Die Pyrolyseprodukte dienten als Modellsubstanzen anhand derer die quantitativen und qualitativen Daten der BPCA-Methode kalibriert wurden. Gegenwärtig werden zwei unterschiedliche Methoden für die Isolierung und den Nachweis von BPCA eingesetzt. Die herkömmliche Methode setzt dazu Gaschromatographie ein (GC-BPCA), die modifizierte Methode benutzt Flüssigkeitschromatographie (LC-BPCA). Die Modellsubstanzen wurden mit beiden Methoden untersucht, wobei deutlich wurde, dass durch LC-BPCA im Vergleich zu GC-BPCA die Reproduzierbarkeit der Ergebnisse verbessert und die Ausbeute von molekularen Markern aus den Modellsubstanzen erhöht wurde. Mit beiden Methoden wurden die höchsten BPCA-Ausbeuten bei mittleren Pyrolysetemperaturen (400-700 °C) ermittelt. Darüber hinaus gibt die BPCA-Methode Auskunft über den Kondensationsgrad in den Verbrennungsrückständen. Die Ausbeute eines Molekularmarkers, B6CA (Mellitsäure), zeigte eine systematische Zunahme bei steigender Pyrolysetemperatur und spiegelte damit den zunehmenden Kondensationsgrad wider.

Weiterhin wurde die BPCA-Methode in dieser Studie auf Umweltproben angewendet, um Veränderungen von Holzkohlen im Laufe ihrer Alterung im Boden zu verfolgen. Dabei konnte beobachtet werden, dass die Löslichkeit von Holzkohle in Wasser äusserst gering ist, dass aber die Löslichkeit mit zunehmender Alterung infolge zunehmender Funktionalisierung erheblich zunimmt. Die Ähnlichkeit in der Verteilung der sieben Markermoleküle zwischen der löslichen Fraktion der Holzkohle und derjenigen, die für gelösten PyC in Flüssen und küstennahen Gewässern festgestellt wurde, legt einen ähnlichen Kondensationsgrad in beiden Fällen nahe. Weiterhin wurden die Veränderungen von PyC-Quantität und -Qualität anhand von landwirtschaftlichen Böden aus West-Kenia untersucht, auf denen es zu bekannten Zeitpunkten zwischen 0 und 100 Jahren vor der Probennahme gebrannt hatte und somit Verbrennungsrückstände deponiert wurden. Entgegen früheren Ergebnissen konnten über die Zeit keine Veränderungen der PyC-Qualität festgestellt werden und auch die PyC-Vorräte im Boden zeigten keinen eindeutigen Rückgang. Die Ergebnisse lassen vermuten, dass sich Holzkohle selbst unter den Bedingungen tropischer Verwitterung gegenüber (bio-)chemischem Abbau sehr resistent verhält.

Mit dieser Arbeit konnte gezeigt werden, dass BPCA ein wertvolles Werkzeug zur umfassenden Beschreibung von PyC in der Umwelt sind, da ein breites Spektrum von Verbrennungsrückstände erfasst wird und zugleich deren Kondensationsgrad abgeschätzt werden kann. Zukünftige Aufgaben beinhalten, die PyC-Qualität in Verbrennungsrückständen aus natürlichen Vegetationsbränden mit derjenigen der laborproduzierten Modellsubstanzen zu vergleichen, Veränderungen von Verbrennungsrückständen durch Abbauprozesse in Laborinkubationsversuche anhand der relativen Verteilung der sieben Markermoleküle zu dokumentieren, sowie die komponentenspezifischen Radiocarbon-Datierung für BPCA-Marker von PyC weiterzuentwickeln.

Summary

Fire residues such as charcoals or soot stem from incomplete combustion during wildfires or anthropogenic burning of fossil fuels and are found ubiquitously in the environment. Pyrogenic carbon (PyC) contained in these fire residues forms a small but significant part of the global carbon cycle. PyC can be described as a continuum of molecules of different degree of aromatic condensation formed when organic matter or biomass is exposed to heating. Generally, the degree of condensation and the stability of PyC are assumed to increase with increasing heat treatment. Because PyC has not a defined chemical structure and comprises a heterogeneous mixture of all kinds of fire-altered materials, qualitative besides quantitative information is needed in order to characterize PyC adequately. Precise estimation of sources, fluxes, and sinks of PyC are needed to come to a global PyC budget. This requires a method which gives reliable results for PyC concentrations in all environmental compartments. Benzene polycarboxylic acids (BPCA), a group of seven molecular markers specific for PyC, are a promising tool for PyC characterization in environmental matrices.

In the scope of this thesis, the BPCA method was systematically tested and two analytical methods for BPCA detection were compared. Charcoals derived from wood (*Castanea sativa* Mill.) and grass (*Oryza sativa* L.) were produced under laboratory conditions at maximum temperatures of 200 °C to 1000 °C. These charcoals served as model compounds to calibrate qualitative and quantitative information provided by the BPCA method. Currently, two methods are used for BPCA isolation and quantification, a traditional method based on gas chromatography (GC-BPCA), and a modified method using liquid chromatography (LC-BPCA). Applying both methods to the model charcoals, LC-BPCA improved reproducibility and increased yields of BPCA molecular markers, compared to GC-BPCA. For both methods, charcoals produced at intermediate temperatures (400-700 °C) gave highest yields of molecular markers. In addition to quantitative information, the BPCA method provides information about the degree of condensation in fire residues. The yields of one of the marker molecules, B6CA (mellitic acid), proved to increase systematically with increasing formation temperature of charcoals, thus reflecting the increasing degree of condensation.

Furthermore, in this study the BPCA method was applied to environmental samples to trace changes of charcoal upon ageing in soils. For solubility of charcoals the results showed that only a small fraction of charcoal is soluble in water, but that the soluble fraction increases strongly with increasing functionalization upon ageing. The similarity in the distribution of the seven BPCA molecular markers suggested a similar degree of condensation of charcoal-derived soluble PyC and molecular structures found in rivers and coastal waters. In a chronosequence of soils which have been converted to agricultural land use by slash-and-burn up to 100 years ago, changes in PyC quantity and quality were investigated. With time the charcoal chemical quality, as measured by BPCA molecular markers for PyC, did not change and charcoal stocks did not show a clear decrease, which is opposite to previous results. The results indicate that charcoal may resist (bio-)chemical degradation even when exposed to intense weathering in a tropical climate.

In this work it could be shown that BPCA are a valuable tool to determine PyC in the environment, as it detects PyC over a broad range of the combustion continuum and simultaneously allows to estimate the degree of aromatic condensation in PyC. Future tasks include comparison of BPCA results obtained for laboratory charcoals with those obtained for charcoals formed during wildfires, analysis of changes in BPCA pattern upon ageing under laboratory conditions and the further development of compound specific radiocarbon dating for BPCA molecular markers.

Contents

Symbols

BPCA	benzene polycarboxylic acids
B3CA, B4CA, B5CA, B6CA	benzenecarboxylic acids with 3, 4, 5 or 6 carboxylic groups, respectively
CSRA	compound specific radiocarbon analysis
GC-BPCA	method that uses gas chromatography for separation of benzene polycarboxylic acids
GC-FID	gas chromatography with flame ionization detection
HPLC-DAD	high performance liquid chromatography with diode array detection
i.d.	inner diameter
LC-BPCA	method that uses high performance liquid chromatography for separation of benzene polycarboxylic acids
m/m	mass fraction
MMM	molecular mixing model
DP NMR	direct polarization nuclear magnetic resonance
PAH	polycyclic aromatic hydrocarbons
PyC	pyrogenic carbon
SD	standard deviation
SE	standard error
TBAB	tetrabutylammonium bromide
TFA	trifluoroacetic acid
Tg	Teragram (10^{12} g)
TOC	total organic carbon
vol. %	volume percent
XPS	X-ray photoelectron spectroscopy

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PART A – Synopsis

1 Introduction

1.1 Fire residues and the role of pyrogenic carbon in the global carbon cycle

Fire residues stem from incomplete combustion during wildfires or anthropogenic burning of fossil fuels. Under optimal combustion conditions carbonaceous materials would be completely converted to CO_2, H_2O and inorganic residues (i.e. ash), but combustion in industrial and natural processes is incomplete under temporary and local limitation of oxygen during the fire event, which leads to the formation of organic fire residues. For forest and savannah fires it was estimated that < 5% and < 3% of the biomass-C consumed by the fire event is converted to fire residues, respectively (Forbes et al., 2006). In absolute numbers, global production of fire residues was calculated to be 50-270 Tg C $year^{-1}$. This includes sources from combustion of biomass in wildfires as well as anthropogenic burning and combustion of fossil fuels (Forbes et al., 2006).

Carbon contained in fire residues, termed pyrogenic carbon (PyC), forms a small part of the global carbon cycle, but is significant for a number of reasons. PyC has been detected in virtually all environmental compartments: in soils (Bird et al., 1999; Schmidt et al., 2001; Glaser & Amelung, 2003; Preston & Schmidt, 2006; Czimczik & Masiello, 2007; Guggenberger et al., 2008), sediments (Smith et al., 1973; Masiello & Druffel, 1998; Schmidt & Noack, 2000; Dickens et al., 2004; Sanchez-Garcia et al., 2010), oceans and rivers (Mitra et al., 2002; Dittmar & Koch, 2006; Dittmar, 2008; Dittmar & Paeng, 2009; Ziolkowski & Druffel, 2010), the atmosphere (Novakov et al., 1974; Horvath, 1993) and in ice cores (Lavanchy et al., 1999). As aerosol in the atmosphere it has a global warming effect by absorbing sunlight (Ramanathan & Carmichael, 2008) and, when deposited on snow it reduces the albedo of the surface, thus contributing to global warming (Hansen & Nazarenko, 2004) and influencing the mass balance of glaciers (Kim et al., 2005; Xu et al., 2009). In deep ocean sediments and soils, PyC can contribute significantly to total organic carbon (TOC) and represents up to 31% and 45% of TOC in sediments and soils, respectively (Masiello & Druffel, 1998; Schmidt et al., 2002). PyC has been found to be among the oldest forms of organic carbon in subsoils (> 10 cm depth), with measured ^{14}C ages of 1,160-9,120 years (Pessenda et al., 2001; Schmidt et al., 2002) and in sediments it was found to be 2,400 to 13,900 ^{14}C years older than non-combusted sedimentary organic carbon deposited at the same time (Masiello & Druffel, 1998). PyC has been suggested as an important carbon sink in the fast atmospheric-biospheric global C cycle (Kuhlbusch & Crutzen, 1995; Kuhlbusch, 1998), but to date much remains unknown about quantification of sources and sinks, transport and transformation of PyC in the environment (Schmidt, 2004; Preston, 2009), which would be important to better assess the role of PyC in the environment. This lack of knowledge complicates a precise estimation of the global PyC cycle (Forbes et al., 2006).

1.2 Properties of pyrogenic carbon

Pyrogenic carbon can be described as a form of carbon which is formed when biomass is exposed to heating. As pyrolysis is a continuous process, it results in a broad variety of compounds with no clear cut boundaries and no single defined structure (Hedges et al., 2000; Schmidt & Noack, 2000; Masiello, 2004). One way to represent the various forms of PyC is a combustion continuum (Hedges et al., 2000), which comprises the whole continuum of fire-altered biomass and organic matter, from slightly charred biomass, to charcoals and soot (Figure 1). In the same order, the intensity of pyrolysis generally increases. Pyrolysis conditions, i.e. temperature, duration, heating rate of pyrolysis and

oxygen content in the atmosphere, have been shown to strongly influence the resulting charcoal properties, such as specific surface area, aromatic carbon content and elemental or isotopic composition (Czimczik et al., 2002; Antal & Gronli, 2003; Krull et al., 2003; Brown et al., 2006; Gundale & DeLuca, 2006). Besides pyrolysis conditions, but to a lesser extent, the properties of the source biomass also influence the resulting charcoal quality (Czimczik et al., 2002; Krull et al., 2003; Gundale & DeLuca, 2006).

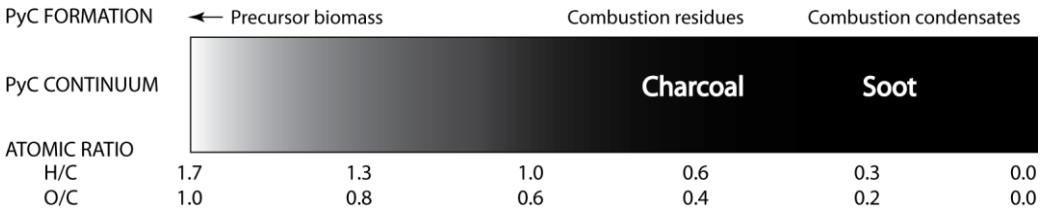

Figure 1: Combustion continuum of pyrogenic carbon (PyC), adapted from Hammes et al., 2007, based on Hedges et al., 2000. Charcoal is the solid residue after a fire event, while soot is formed as a recondensate from the gas phase. Atomic H/C and O/C ratios decrease as pyrolysis intensity increases (from left to right).

The processes associated with biomass heating lead to loss of H, C and O into the gas phase in form of H_2O, CO, CO_2 and CH_4, resulting in decreasing H/C and O/C ratios compared to the initial biomass (Baldock & Smernik, 2002; Kim et al., 2003; Braadbaart et al., 2004; Hammes et al., 2006) (Figure 1). The elemental changes are indicative for a rearrangement of organic carbon and a transformation of relatively labile compounds into more recalcitrant aromatic structures of different degrees of condensation (Shafizadeh & Sekiguchi, 1983; Knicker et al., 1996; Baldock & Smernik, 2002; Czimczik et al., 2002). Consequently, all PyC has one chemical feature in common: the presence of condensed aromatic ring structures, which are formed upon heating. These condensed aromatic structures are assumed to be highly recalcitrant, as suggested by the high resistance to a range of chemical oxidants (Skjemstad et al., 1996; Bird & Gröcke, 1997; Ascough et al., 2011). On the contrary, incubation studies have demonstrated the general ability of microorganisms to degrade even these very stable forms of carbon (Hamer et al., 2004; Brodowski, 2005; Kuzyakov et al., 2009). However, observed degradation rates are much lower for PyC compared to the degradation rates of the source biomass (Baldock & Smernik, 2002) and increasing the heat treatment temperature of the biomass leads to a decrease of PyC degradability, which is attributed to the increasing degree of condensation at higher temperatures and resulting higher resistance against microbial degradation (Baldock & Smernik, 2002; Hamer et al., 2004; Bruun et al., 2008; Nguyen & Lehmann, 2009; Nguyen et al., 2010; Zimmerman, 2010). On the other hand, recalcitrance of PyC against degradation has been questioned by studies showing substantial losses of PyC from the soil profile over time (Bird et al., 1999; Hammes et al., 2008b; Nguyen et al., 2008) or transformation and mineralization of PyC in incubation studies (Hilscher et al., 2009; Hilscher & Knicker, 2011; Knicker, 2011). One reason for the contradicting results could be different forms of PyC used in the respective studies, i.e. less vs. more condensed aromatic PyC. The contradictory results on PyC stability underline the need for qualitative in addition to quantitative information on PyC, in order to assess its role in the environment more adequately.

1.3 Measuring pyrogenic carbon- specification of analytical challenges

One of the major challenges in PyC research is the fact that PyC has not a defined chemical structure but represents a group of substances which were exposed to elevated temperatures. As a consequence of the heterogeneous character of PyC and its presence in multiple environmental compartments, several methods were developed for its quantification, of which seven common methods were assessed in a multi-method, multi-laboratory comparison study (Hammes et al., 2007). None of the methods used to measure PyC was found to be capable of covering the whole spectrum of pyrogenic molecules occurring in the combustion continuum. Each method has an analytical window specific for the respective method and the detection efficiency of each method was found to vary across the PyC continuum. In order to know more about what is actually measured by a particular method, it is important to define the analytical window of the respective method in detail. This is necessary to interpret the results obtained for samples from soils or sediments adequately.

The present study focuses on one of the tested methods, which uses molecular markers to determine PyC. Compared to other methods used for PyC measurements, the molecular marker method shows two major advantages. First, and in contrast to other methods, it measures molecular markers which are specific for condensed aromatic ring structures typically occurring in PyC (Glaser et al., 1998). Thus, it adds a chemical definition to PyC, whereas other methods report quantities of an operationally defined, oxidation-resistant subfraction of organic carbon. Second, it measures a group of marker molecules and their relative contribution can be related to source and formation conditions of PyC, thus yielding quantitative and with the same analytical procedure qualitative information (Glaser et al., 1998; Brodowski et al., 2005b; Dittmar, 2008; Guggenberger et al., 2008; Hammes et al., 2008b; Ziolkowski & Druffel, 2009a; Ziolkowski & Druffel, 2010). This information can be of great use in order to get deeper insight into the different forms of PyC in the combustion continuum. However, there are also some known disadvantages of this method, as pointed out by Hammes et al. (2007): It has been found that the method slightly underestimates highly condensed aromatic forms of PyC such as soot. Further, the method showed a high inter-laboratory variability with a factor of two or more for six of the twelve samples analyzed in the comparative study, i.e. laboratories using nominally the same method produced different results on the same samples, which can probably be attributed to the many sample treatment steps involved in this method. Also, the method is prone to positive biases from non-pyrogenic materials, such as shale (average 4.1% PyC of TOC) and coals (15.6-20.9% PyC of TOC), which must be kept in mind when the method is applied to samples which might contain such forms of organic carbon. Hence, the objectives of this study were (i) to make use of the unique possibilities offered by the molecular marker method and (ii), to reduce the associated uncertainties.

2 Objectives

Precise estimation of sources, fluxes, and sinks of PyC are needed to come to a global PyC budget. This requires a method which gives reliable results for PyC concentrations in all environmental compartments. The objective of this thesis is to develop an analytical tool, which gives reliable information about quantity and quality of fire residues in the environment, in order to facilitate the calculation of a global PyC budget in the future. This study can contribute to a better understanding of the use of molecular markers for the characterization of pyrogenic C in the environment.
More specifically, the work presented in this thesis will improve the understanding of how the PyC component of charcoals is represented by molecular markers and will take advantage of molecular markers ability to trace the fate of PyC in soils upon ageing.

Questions that will be answered in this thesis:

1. Can we simplify the molecular marker method and still get the same qualitative and quantitative results?

Besides the traditional molecular marker method (Glaser et al., 1998; Brodowski et al., 2005b), a refined method has recently been presented (Dittmar, 2008) omitting most of the sample preparation steps by using liquid instead of gas chromatography for separation and measurement of marker molecules. **Hypothesis:** The modified detection method with less preparation and cleaning steps will give higher yields of the molecular markers from a suite of standard charcoals compared to the traditional method.

2. Does the molecular marker method for pyrogenic carbon systematically reflect qualitative changes in charcoals produced at different temperatures?

There are indications from earlier studies that mellitic acid (B6CA) as detected by the molecular marker method can provide information about the degree of condensation of charcoals (Hammes et al., 2008b). Heat treatment temperature has been shown to have a strong influence on many physical and chemical properties of charcoal (Shafizadeh & Sekiguchi, 1983; Brown et al., 2006). The feedstock properties influence charcoal quality to a lesser extent (Czimczik et al., 2002; Brown et al., 2006; Gundale & DeLuca, 2006). **Hypothesis:** The relative contribution of the individual marker molecules changes systematically and independently from the source biomass with increasing heat treatment temperature towards higher contribution of mellitic acid (B6CA).

3. Which part of the combustion continuum is covered by the molecular marker method?

Formation of aromatic structures typically occur at temperatures > 300 °C (Shafizadeh & Sekiguchi, 1983), thus in low temperature charcoals the content of aromatic structures might be too low to be detected by the molecular marker method. For the other end of the PyC continuum it has been reported that highly condensed aromatic structures such as soot are underestimated by the method (Hammes et al., 2007). One aim of the present study is to describe the analytical window of the molecular marker method in more detail. **Hypothesis:** The method produces highest yields at intermediate heat treatment temperatures (300-700 °C), while lowest (< 300 °C) and highest (> 700-1000 °C) heat treatment temperatures results in lower yields of molecular markers.

4. Which fraction of freshly produced and aged charcoal gets solubilized in water?

As yet much remains unknown about transport of PyC in the environment. In soils one possible pathway for export of PyC is in dissolved or colloidal form into deeper soil horizons (Hockaday et al., 2006; Hockaday et al., 2007). **Hypothesis:** Small units of condensed aromatic structure are preferentially found in the dissolved or colloidal fraction of PyC and this fraction increases with increasing residence time of charcoal in the soil.

5. Are chemical changes of charcoals upon weathering in a tropical climate reflected in the molecular marker qualitative information?

There are indications that the molecular marker contributions change upon degradation of charcoals both, under field conditions on longer time scales up to 100 years (Hammes et al., 2008b) and in short-term laboratory incubation studies (Brodowski, 2005). On the other hand, charcoals are known for their resistance against degradation and observed chemical changes might be restricted to the surface of macroscopic charcoals particles (Nguyen et al., 2008). **Hypothesis:** With increasing time of deposition in a tropical soil less stable forms of charcoal are preferentially degraded while more stable forms become relatively enriched, resulting in changing relative contributions of the individual marker molecules.

3 Experimental

3.1 Benzene polycarboxylic acid method

A molecular marker method was used in this study to measure the PyC component in soils (section 3.2) and charcoals (section 3.3). The method used here is based on the benzene polycarboxylic acid (BPCA) method described by Glaser et al. (1998) with modifications introduced by Brodowski et al. (2005b). BPCA are molecular markers specific for condensed aromatic structures typically occurring in fire-derived organic materials (Glaser et al., 1998). The principle of the method is based on the conversion of the condensed structures into benzene carboxylic acids through oxidation treatment using 65% (m/m) HNO_3 at 170 °C for 8 hours (Figure 2), which subsequently can be separated and quantified via gas chromatography and flame ionization detection (GC-FID).

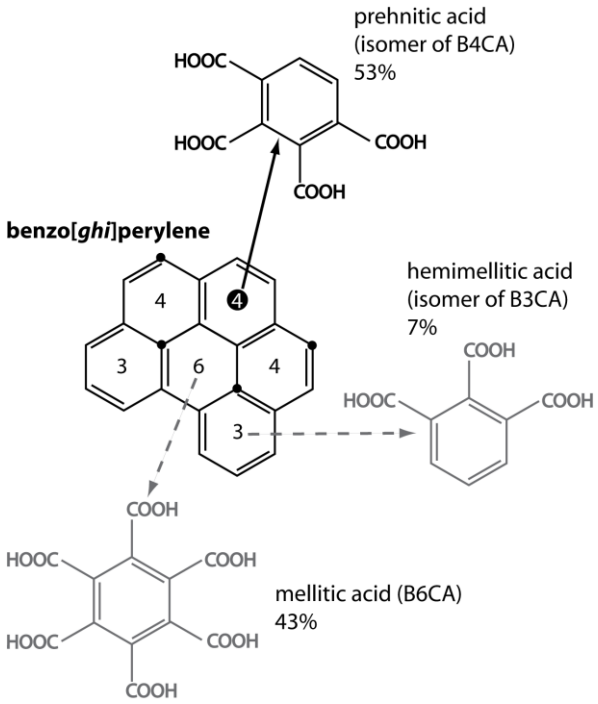

Figure 2: Principle of production of benzene polycarboxylic acids (BPCA) from condensed aromatic molecules (based on Dittmar, 2008; modified with data from Ziolkowski, 2009). As an example the molecular structure of benzo[*ghi*]perylene is shown. The numbers in the rings denote the number of carboxylic substitutions if the respective ring is oxidized to BPCA. It must be noted that per molecule benzo[*ghi*]perylene only one BPCA molecule can be produced. The black circled number gives an example for one ring that is oxidized to BPCA, with the black dots marking the positions, which are oxidized to carboxylic acids. The product is prehnitic acid, which is the most abundant BPCA derived from benzo[*ghi*]perylene (53% of total BPCA-C). The production of B6CA (40%) and B3CA (7%) is also possible (shown grey in the figure). The total C recovery from benzo[*ghi*]perylene in form of BPCA-C is 22.5% (±3.9% SD) (Ziolkowski, 2009).

In the following chapters, the BPCA isomers substituted with three carboxylic groups (hemimellitic acid and trimellitic acid) are summed up and quantified as “B3CA”, and those with four carboxylic groups (pyromellitic acid, mellophanic acid, prehnitic acid) are summed up and quantified as “B4CA”. Benzenepentacarboxylic acid will be referred to as “B5CA” and mellitic acid as “B6CA”. Trimesic acid, another isomer with three carboxylic groups, is not used as a marker for PyC, because its origin cannot be related unambiguously to polycondensed aromatic, pyrogenic structures (Haumaier, 2010).

The method includes various cleaning procedures, basically all of them aiming at elimination of polyvalent cations (Al, Fe). The cations interfere with the synthesis of volatile trimethylsilyl derivatives of the target compounds, and thus hinder the further analysis using gas chromatography.
An internal standard is used to monitor losses during sample preparation. Citric acid, previously used as internal standard, was shown to deteriorate under strongly acidic conditions typically occurring during sample clean up. Therefore, we changed the protocol of the method and citric acid was replaced by phthalic acid, which is stable at pH < 0.7 (Manuscript 1). To ensure comparability with previous results, the reference charcoals produced at 450 °C from the comparative study of PyC methods (Hammes et al., 2006; Hammes et al., 2007; Hammes et al., 2008a) were re-analyzed with the current method. The BPCA yields reported in the ring trial study were reproducible for two of the three participating laboratories analyzing the wood charcoal and for all data on grass charcoal (data: Part C - Appendix).
The BPCA method has been recently modified for PyC analysis of seawater samples using an alternative possibility for the separation and detection of BPCA (Dittmar, 2008). This modified method employs high performance liquid chromatography and diode array detection (HPLC-DAD) instead of GC-FID. The use of HPLC-DAD has several advantages compared to GC-FID (Dittmar, 2008): For the modified method, most sample preparation steps necessary for GC-FID can be omitted. First, no derivatization is necessary as samples are analyzed directly in liquid state. Second, also other treatment steps can be omitted using HPLC-DAD, making analysis less time consuming and less cumbersome. In the following, the two detection methods will be referred to as GC-BPCA for the traditional method using gas chromatography and as LC-BPCA for the modified method using high performance liquid chromatography. An overview of both detection methods and the respective sample preparation steps can be found in Table 1.

Table 1: Specifications for sample preparation steps and for chromatographic analysis of benzene polycarboxylic acids (BPCA), using either gas chromatography with flame ionization detector (GC-BPCA) or high performance liquid chromatography with diode array detector (LC-BPCA) for BPCA separation and quantification (adapted from Manuscript 2). For details please see laboratory protocols in Part C – Appendix.

work step/ description	Traditional (GC-BPCA) (Glaser et al., 1998; Brodowski et al., 2005b; Schneider et al., 2010)	Modified (LC-BPCA) (Dittmar, 2008; Schneider et al., 2011)
1. Sample preparation before HNO_3 digestion	trifluoroacetic acid (TFA) digestion, filtration, drying at 40 °C	–
2. Conversion to BPCA	addition of 2 mL 65% HNO_3 (8 hours at 170 °C in oven)	
3. Sample preparation after HNO_3 digestion	filtration over ashless cellulose filter into 10 mL volumetric flasks, fill up with deionized water	drying at 60 °C under N_2 stream and dissolution in methanol/ water, transfer to LC vials
4. Removal of cations	addition of internal standard, cleaning with cation exchange resin, freeze drying, transfer to GC vials	–
5. Derivatization	100 µL BSTFA+TMCS, 100 µL pyridine	–
6. Chromatographic analysis	Gas chromatography with flame ionization detection (GC-FID)	High performance liquid chromatography with diode array detection (HPLC-DAD)
7. Identification	retention time, gas chromatography with mass spectrometry (GC-MS)	retention time, absorbance spectra 220-380 nm
Standard error for BPCA-C kg^{-1} OC in thermosequence charcoals [%]	mean: 6.5; min.: 1.3; max.: 34.8	mean: 1.6; min.: 0.3; max.: 4.5

3.2 Soils

3.2.1 Chernozem soil used as reference material

The Haplic Chernozem (FAO-UNESCO, 1998) was sampled in the region of Hildesheim-Braunschweig at Harsum (northern Germany) at 20-60 cm depth, to minimize anthropogenic influence from ploughing and bomb ^{14}C. The soil has 19% clay content and 53% sand content, and an organic carbon content of 20.1 g OC kg^{-1} soil. The material has been dried, sieved < 2 mm, homogenized and sterilized with gamma radiation. The soil was used before to compare different quantification methods of PyC (Hammes et al., 2007) and has been described in detail by Schmidt et al. (1999). Here it was used to investigate the reproducibility of results for soil samples obtained by the molecular marker method (Manuscript 1).

3.2.2 Soil chronosequence from Kenya

The soils used in the study about long-term stability (Manuscript 4) come from a chronosequence from western Kenya and were classified as Humic Nitosols (FAO-UNESCO, 1998), which were deep dark reddish brown soils with friable clay and thick humic topsoils with 45-49% clay, 15-25% silt, and 26-40% sand (Solomon et al., 2007; Kimetu et al., 2008; Kinyangi, 2008). Slash and burn had been used at the experimental sites to convert the native forest to cultivated soils during the past 100 years. Forest trees were cut and burnt on site during conversion. After conversion, the soil was plowed to 0.1-0.12 m depth for maize (*Zea mays* L.) cultivation. Burning was not practiced on fields

following initial conversion. Therefore, ages of the last PyC input correspond to the time since the conversion from forest to agriculture, making use of different conversion ages to construct a time series or chronosequence of PyC ages (Solomon et al., 2007; Kimetu et al., 2008; Nguyen et al., 2008). A subset of the soil samples from southern Nandi forest series was selected for this study to examine changes in PyC over time.

3.3 Charcoals

3.3.1 Production of thermosequence charcoals

The formation of charcoals has been often described as one of the important sources of PyC in the environment (Kuhbusch & Crutzen, 1996; Preston & Schmidt, 2006). Charcoals were therefore chosen as model compounds for PyC analysis (Manuscripts 1 and 2). Charcoals differ in their properties depending on source materials and formation conditions, especially on temperature of formation (Shafizadeh & Sekiguchi, 1983; Brown et al., 2006). In order to obtain material, which is suited to assess the performance of the molecular marker method over a broad range of the combustion continuum, charcoals were produced from grass and wood at maximum temperatures of 200 °C to 1000 °C. Pyrolysis was conducted under well-defined laboratory conditions in order to guarantee reproducibility, following the protocol of Hammes et al. (2006). A tube furnace (Carbolite CTF 16/75, Sheffield, UK) was used for production of charcoals under constant N_2 flow of 13 L hour^{-1} and with a heating ramp rate of 300 °C hour^{-1} up to 200 °C, followed by a ramp of 50 °C hour^{-1} until the final temperature was reached. The respective maximum temperature was held constant for 5 hours to ensure complete charring. We used small wood (*Castanea sativa* Mill.) chips and cut rice grass (*Oryza sativa* L.) pieces as two important biomass sources of charcoal in the environment, representing lignin-rich (woody tissue) and lignin-poor (grass straw) sources. Further, compared to woody tissues, grass can contain considerable amount of silica phytoliths (Tsartsidou et al., 2007) and organic matter occluded in these phytoliths (Krull et al., 2003), which can influence the pyrolysis process and resulting charcoal quality, such as elemental composition (Raveendran et al., 1995).

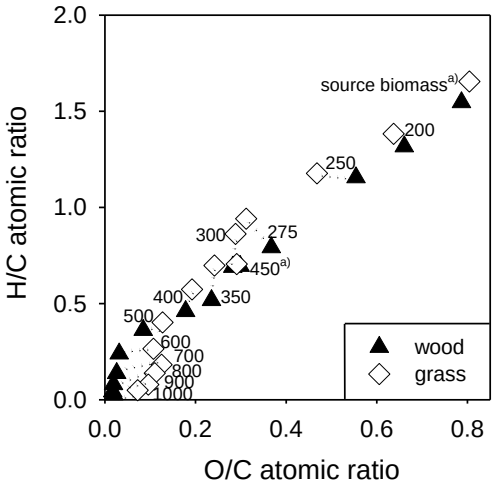

Figure 3: Atomic ratios (O/C and H/C) of pyrolyzed chestnut wood (*Castanea sativa* Mill.) and rice grass (*Oryza sativa* L.), taken from Manuscript 2. Grass and wood samples pyrolyzed at the same temperature are connected with a dotted line, numbers indicate the respective pyrolysis temperatures (200-1000 °C). Included is elemental data for the untreated source biomass and reference charcoals produced at 450 °C; a) data taken from Hammes et al. (2006).

Atomic O/C and H/C ratios of the charcoals can be found in Figure 3. In the following sections this series of 24 charcoals will be referred to as “thermosequence of charcoals”. These charcoals produced under well-defined conditions serve as model substances to test the quantitative and qualitative information on the pyrogenic carbon fraction provided by the molecular marker method.

3.3.2 Origin of aged charcoal

Aged charcoal was obtained from a prescribed burn experiment, which took place on 28 March 1998. The experiment was conducted in a sweet chestnut (*Castanea sativa* Mill.) stand in southern Switzerland (San Antonino, Canton Ticino). The fuel load at the sampling site was 2.3 kg m^{-2}, and the maximum temperature during the fire event in the fuel layer was 487 °C (Wüthrich et al., 2002). Samples were collected in May 2009. These samples were exposed to biotic and abiotic oxidative processes in the soil for more than 10 years and are assumed to be partially oxidized by degradation.

3.4 Isolation of soluble and colloidal fractions from fresh and aged charcoal

Soluble and colloidal fractions were obtained from a standard charcoal pyrolized at 450 °C (Hammes et al., 2006) and an aged charcoal from a prescribed burn experiment (section 3.3.2). To isolate soluble and colloidal fractions of charcoals 8 and 4 g, respectively, of ground charcoal material was added to 100 mL of water, shaken for 6 hours and after that centrifuged at 4000 rpm. Subsequently, the samples were filtered over membrane filter papers of 0.45 µm (Whatman ME25, Schleicher & Schuell, Dassel, Germany, soluble fraction) or 5 µm pore size (Whatman AE98, colloidal fraction), freeze dried and analyzed for BPCA using GC-FID as described above (section 3.1). In order to obtain enough material of the soluble fraction for the molecular marker analysis, the experiment was conducted in six replicates and samples were combined into one composite sample.

4 Discussion

In the following subsections, results for pyrogenic carbon (PyC) molecular markers of the manuscripts are highlighted to discuss differences in the results obtained by two detection methods for BPCA (section 4.1), to specifically analyze the information provided by the BPCA method on quality (section 4.2) and quantity (section 4.3) of PyC in charcoals, and to investigate properties of the soluble fraction of charcoals (section 4.4) and changes in charcoal quality upon degradation in soils (section 4.5) as detected by the molecular marker method.

4.1 A modified BPCA method using liquid chromatography improves reproducibility and increases yields of molecular markers

Benzene polycarboxylic acids (BPCA) have been used widely to analyze the PyC component in soils (Glaser et al., 2000; Czimczik et al., 2003; Glaser & Amelung, 2003; Rodionov et al., 2006; Brodowski et al., 2007; Guggenberger et al., 2008; Hammes et al., 2008b; Rodionov et al., 2010), sediments (Sanchez-Garcia, 2007), and charcoals (Kaal et al., 2008). Recently, a modification of the BPCA method was introduced for the determination of PyC in ocean water (Dittmar, 2008). This modified method employs liquid chromatography (LC-BPCA) instead of gas chromatography (GC-BPCA) for isolation of the molecular markers, which greatly simplifies sample preparation (Table 1). Due to the differences in sample preparation and detection method as yet it is not clear if both methods would yield same results of molecular markers in different samples, but this would be important in order to compare PyC data obtained from terrestrial and marine samples. Here a charcoal thermosequence made from wood and grass was used as standard material for a comparison of the two BPCA methods.

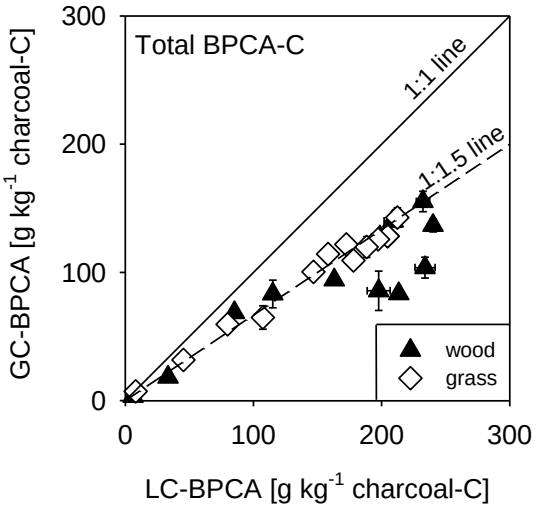

Figure 4: Comparison of marker molecule yields (total BPCA-C per charcoal-C) in thermosequence charcoals as obtained by two different methods, using either high performance liquid chromatography with diode array detection (LC-BPCA, x-axis) or gas chromatography with flame ionization detector (GC-BPCA, y-axis). The thermosequence comprises charcoals derived from two sources of biomass, chestnut wood (*Castanea sativa* Mill.) and rice grass (*Oryza sativa* L.), pyrolyzed at 12 temperatures from 200 to 1000 °C. For easier comparison the 1:1 (solid) and 1:1.5 line (dashed) are shown (data taken from Manuscript 2).

Results from the study show that yields of BPCA for intermediate temperature charcoals (300-800 °C) were systematically 1.5±0.3 times higher when using the modified, LC-based method (Figure 4). For lowest and highest temperature charcoals even higher differences have been observed. Further, it was demonstrated that the standard error between laboratory replicates could be reduced, on average from 6.5% to 1.6% when using LC-BPCA instead of GC-BPCA (Table 1). However, all of the marker molecules (B3CA, B4CA, B5CA, B6CA) showed a similar reduction in their yields, and thus, no bias towards more or less carboxylated markers could be detected in one or the other method. This means that both methods equally are able to detect qualitative differences in the degree of condensation.

The quantitative differences observed are attributable to the differences in the sample preparation procedure. In the filtration step following nitric acid oxidation (Step 3 in Table 1) we observed losses of about 20% of BPCA when tested with a standard mixture. Losses of up to 10% have also been reported for the use of the cation exchange resin (Step 4 in Table 1) when analyzing soot and carbon nanotubes for BPCA (Ziolkowski & Druffel, 2009a). Both observations together could explain large part of the quantitative differences.

In Manuscript 2 we propose several measures to improve sample processing for GC-BPCA. Losses in the filtration step could be reduced by increasing the volume to rinse the filter from 8 mL to 48 mL. Also, the use of polycyclic aromatic hydrocarbons (PAH) such as anthracene as a test material to monitor losses during the whole sample cleaning procedure for GC-FID analysis could help to quantify the losses more accurately. However, these measures have not yet been applied and would require additional testing if they are suitable to reduce or correct for losses.

In order to compare results obtained for PyC in soils by the traditional GC-BPCA method with those obtained for water samples by the modified method (LC-BPCA) we propose a correction factor of 1.5 in the study. For the future it is suggested to implement the LC-based method for all types of samples from diverse environmental matrices in order to reduce losses and sample handling and to improve reproducibility and comparability of PyC measurements in soils, sediments and waters.

In the following sections, data was obtained by LC-BPCA for thermosequence charcoals (sections 4.2 and 4.3), while for environmental matrices the GC-BPCA method was used (sections 4.4 and 4.5).

4.2 Mellitic acid (B6CA) serves as an indicator of the formation temperature of charcoals

The degree of condensation is an important measure for the description of PyC along the combustion continuum gradient and is assumed to increase with increasing intensity of the thermal impact on biomass. Further, it might influence the resistance of PyC against biotic and abiotic degradation (Zimmerman, 2010), and thus, its longevity in the environment. It is an important advantage of the chosen method that besides quantitative information it provides additional information about the quality of the tested materials via the relative distribution of the seven marker molecules. However, as yet it is not clear if the relative contribution of the marker molecules can be directly linked to a certain heat treatment temperature.

In order to get greater insight into the mechanism of conversion of the condensed aromatic clusters into BPCA, several studies were conducted using PAH as model compounds for BPCA production (Dittmar, 2008; Ziolkowski, 2009). Generally it was shown that the production of B3CA and B4CA requires minimum condensed units of 3 aromatic rings (e.g. retene), whereas the production of B6CA requires a minimum of 5 condensed aromatic rings (e.g. perylene). Glaser et al. (1998) found that B6CA is only formed when pyrolysis was conducted for more than 15 min (at 300 °C), while for

shorter exposure time B6CA was absent in the HNO_3 digest. Further it was demonstrated that materials known to contain highly condensed forms of PyC, such as activated charcoals (Dittmar, 2008) or soot and fullerenes (Ziolkowski & Druffel, 2009a), preferentially yield B6CA molecular marker with about 70% (activated charcoal and soot) and 90% (fullerene) relative contribution of B6CA-C to total BPCA-C . Thus it can be assumed that an increasing contribution of B6CA indicates increasing cluster sizes of condensed aromatic structures, which are indicative for more intense pyrolysis conditions of the respective material.

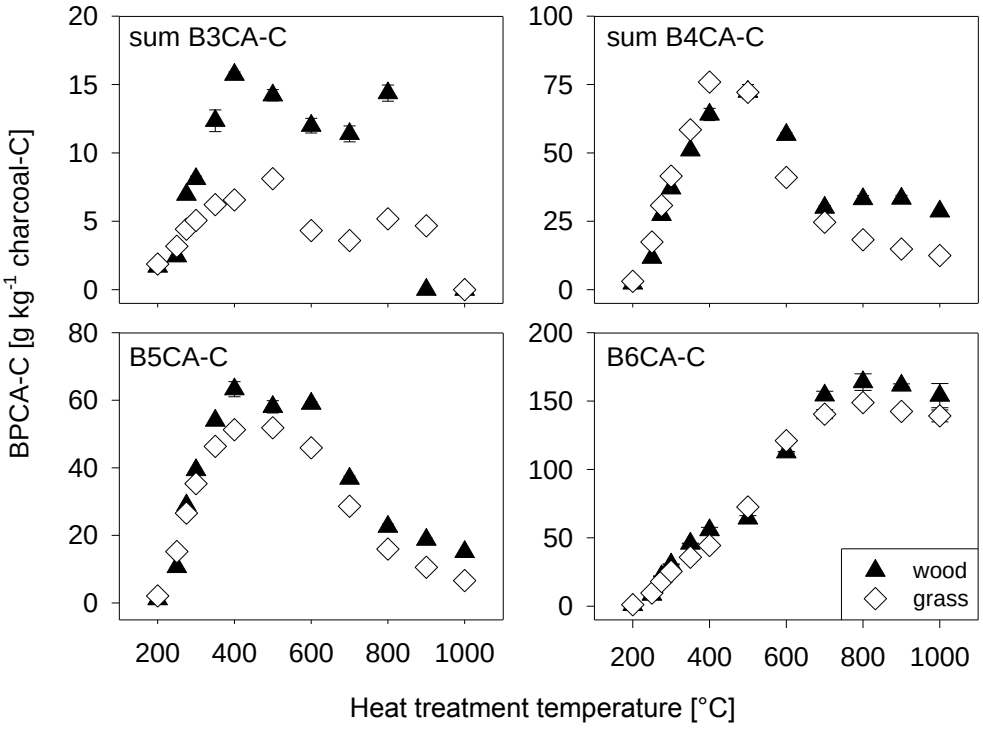

Figure 5: Yields of B3CA (sum), B4CA (sum), B5CA and B6CA from chestnut wood (*Castanea sativa* Mill., triangles) and rice grass (*Oryza sativa* L., diamonds) thermosequence charcoals with heat treatment temperatures from 200-1000 °C, normalized to charcoal carbon content, based on data from Manuscript 2. Please note the different scaling of the y-axis.

Looking at the contribution of the individual marker molecules derived from the thermosequence charcoals (Figure 5) it becomes clear that B3CA, B4CA and B5CA in the lower temperature range strongly increase, but as heating moves beyond 500 °C these marker molecules show a sharp decrease, whereas B6CA constantly increases. Only for the highest temperatures 900 and 1000 °C there is no further increase and B6CA remains constant. The observed trends are same for wood and grass charcoals, indicating a dominant influence of the heat treatment temperature and less influence of properties of the starting material on the distribution of the marker molecules. The increase in B6CA on the expense of the other markers at temperatures > 500 °C can be interpreted as an increase in degree of condensation due to the growth of graphene-like structures at these temperatures. A pronounced increase in aromaticity and condensation of aromatic rings in charcoals at temperatures > 600 °C was also observed using X-ray photoelectron spectroscopy (Nishimiya et al., 1998).

4.3 Charcoals produced at intermediate temperatures give highest yields of molecular markers

If we sum up the carbon contained in the individual markers B3CA, B4CA, B5CA and B6CA we get the total BPCA-C yields (Figure 6), which is used as the quantitative measure of PyC. For wood and grass charcoals, a sharp increase in total BPCA-C yields could be observed in the temperature range of 200-400 °C from 6.0 g kg^{-1} OC to 198.7 g kg^{-1} OC for wood, and from 8.1 g kg^{-1} OC to 178.1 g kg^{-1} OC for grass charcoals (Figure 6), followed by a maximum at 600 °C for both source biomasses (240.2 g kg^{-1} in wood and 212.2 g kg^{-1} in grass charcoals). The results can be attributed to the formation of aromatic carbon upon heating. Increasing aromaticity upon heating of biomass has been reported by others before using elemental (Shafizadeh & Sekiguchi, 1983; Antal & Gronli, 2003) and spectroscopic (Knicker et al., 1996; Czimczik et al., 2002; Smernik et al., 2006) analytic methods. At higher temperatures (800-1000 °C) a slight decrease in the BPCA-C yields can be observed, despite decreasing H/C ratios suggest ongoing condensation (Figure 3). At high temperatures (600-1000 °C) there is a higher yield of BPCA for wood charcoals compared to grass charcoals, which is attributable to the higher content in aromatic precursor molecules in wood such as lignin (Mok et al., 1992; Czimczik et al., 2002). But differences between grass and wood are generally small and total BPCA yields show similar trends for both sources of biomass.

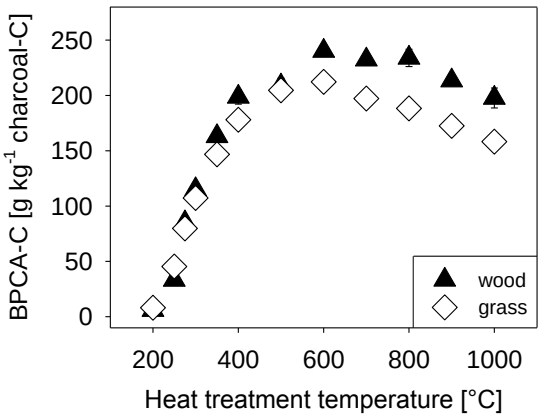

Figure 6: Total BPCA yields from chestnut wood (*Castanea sativa* Mill., triangles) and rice grass (*Oryza sativa* L., diamonds) thermosequence charcoals with heat treatment temperatures from 200-1000 °C, normalized to charcoal carbon content (based on data from Manuscript 2).

Looking at the total BPCA yields it becomes clear that not even half of charcoal carbon is recovered as BPCA-C. For each BPCA molecule produced from a condensed aromatic structure there will be losses of C because neighboring aromatic rings are destroyed and C of these ring is only partially quantified in form of carboxylic groups attached to the benzene ring of the produced BPCA molecule (Figure 2). These losses during the conversion to BPCA by HNO_3 oxidation of the material, e.g. in form of CO_2, are inherent to the conversion process. Some other fraction of the initial material is converted to aromatic compounds containing nitrous functional groups, which are not accounted for (Ziolkowski, 2009). This explains why the maximum conversion of charcoal-C into BPCA-C is only about 25% of total organic C (Figure 6), although the intensively pyrolized material kept 5 hours at the respective maximum temperature is assumed to contain 100% PyC by definition. Thus, it should

be kept in mind when interpreting BPCA data that BPCA-C must be considered a semi-quantitative measure of PyC, which gives a conservative estimate of PyC content in the sample.

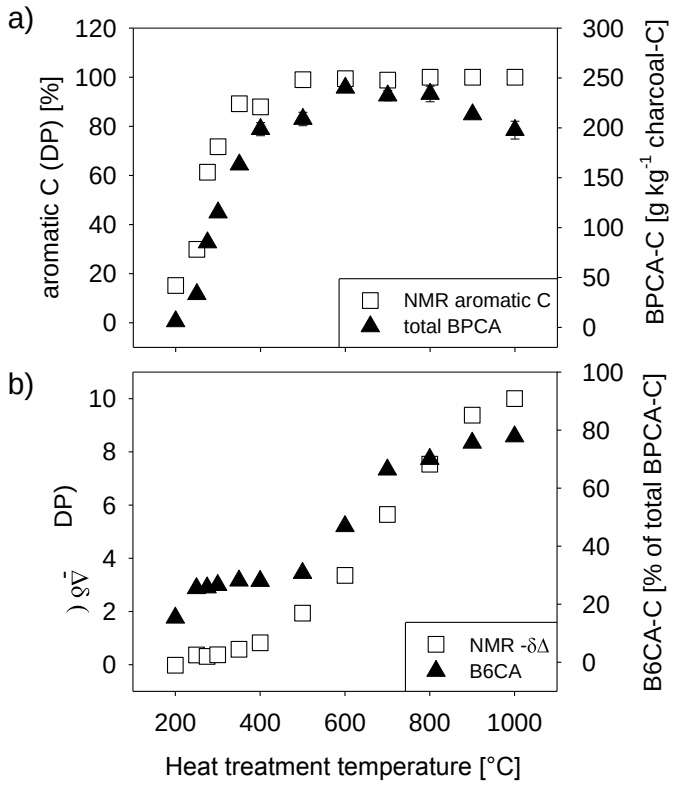

Figure 7: Both graphs show data for the chestnut (*Castanea sativa* Mill.) wood thermosequence with heat treatment temperatures from 200-1000 °C. a) Comparing the measure of % aromatic C obtained by ^{13}C direct polarization nuclear magnetic resonance (DP NMR) spectroscopy (open squares) with the total benzene polycarboxylic acid (BPCA) yields (black triangles) b) Comparing ^{13}C DP NMR-based ring current Δδ measurements (open squares) with the relative contribution of B6CA to total BPCA yield (black triangles) (from McBeath et al., 2011).

How does the information obtained by the BPCA method fit into the understanding of the pyrolysis processes which we get from other analytic methods? Recently, the wood charcoal thermosequence, which was used for the present study, has been analyzed using ^{13}C direct polarization nuclear magnetic resonance (DP NMR) spectroscopy (McBeath et al., 2011). The ^{13}C NMR spectroscopy provides two parameters that give similar information as two parameters provided by the BPCA method: the total BPCA-C yield (g kg^{-1} OC) is comparable to the NMR measure of percent aromatic C and the relative proportion of B6CA-C in total BPCA-C is comparable to the NMR measure of chemical shift of ^{13}C-labeled benzene sorbed to the charcoals (called Δδ). A shift towards lower ppm values can be attributed to stronger diamagnetic ring currents, which are indicative for a higher degree of condensation (Smernik et al., 2006; McBeath & Smernik, 2009). Figure 7a shows that there is a close correlation between total BPCA-C yield (g kg^{-1} OC) and NMR-determined charcoal aromaticity over the whole temperature range 200-1000 °C. The close correlation indicates that a similar proportion of charcoal aromatic C is converted to BPCA molecular markers through this temperature range. Figure 7b shows there is also a general correlation between the relative proportion of B6CA and Δδ, both of which indicate increasing degree of condensation especially at temperatures > 500 °C. The

good correlation to the ^{13}C NMR spectroscopic data provides additional support for the plausibility of the results obtained by the BPCA method.

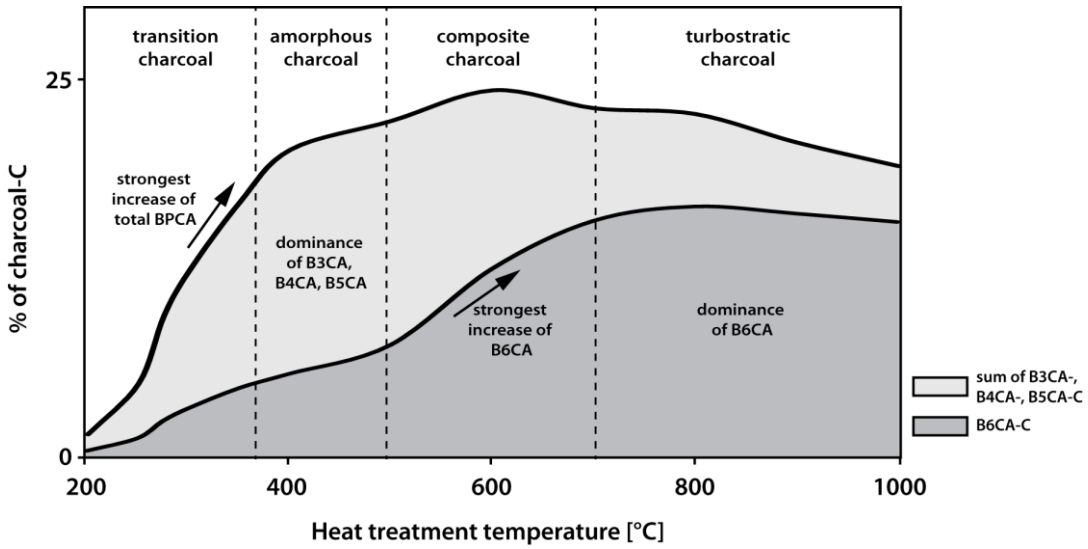

Figure 8: Different types of charcoal formed along a temperature gradient and corresponding changes in total BPCA and B6CA yields (adapted from Keiluweit et al. (2010) and modified with own data). The difference between total BPCA and B6CA yield is the sum of the other marker molecules B3CA, B4CA and B5CA (light grey area). A dominance of B3CA, B4CA and B5CA is indicative for small cluster sizes of 4-6 condensed aromatic rings, whereas the dominance of B6CA indicates growth of highly condensed, graphene-like structures. The curves are based on the mean BPCA yields obtained from wood and grass charcoal pyrolized at the same temperature.

A conceptual model has recently been proposed to describe changes in biomass and formation of PyC upon heating (Keiluweit et al., 2010). In the following, the processes and types of charcoal described there are related to the BPCA results obtained for quantity and quality of PyC in the thermosequence charcoals (Figure 8). The increase in total BPCA-C yields observed at temperatures 200-375 °C indicates an increasing contribution of condensed aromatic moieties in transition charcoals, probably accompanied by a loss of properties of the original biomass (Keiluweit et al., 2010). As temperature increases, the slightly charred plant material is further transformed to amorphous charcoals. The dominance of B3CA, B4CA, and B5CA indicates small aromatic cluster sizes and can be related to the formation of pyrogenic amorphous carbon. These small aromatic units arranged in random order dominate the amorphous charcoals formed at temperatures 375-500 °C. Here, the increase in total BPCA yield starts to level off. As temperatures rises to higher temperatures of 500-700 °C, the yields of B3-B5CA start to decrease, while we find a pronounced increase in B6CA yields (Figure 8). This reflects the increasing growth of more condensed structures observed in composite charcoal (Keiluweit et al., 2010). The dominance of B6CA in charcoals formed at temperatures of 700-1000 °C is indicative for highly condensed, graphene-like structures in turbostratic charcoals (Nishimiya et al., 1998), which are made up from stacks of graphene sheets in a disordered matrix (Franklin, 1951).
Compiling all qualitative and quantitative BPCA data on the charcoal thermosequence from sections 4.2 and 4.3, it appears that pyrolysis driven processes occurring in biomass upon heating are systematically reflected by BPCA molecular markers.

4.4 Only a small fraction of charcoal gets solubilized in water

Dissolved organic matter is an important fraction of organic materials in many soils, because it represents a highly mobile fraction and thus it can influence the distribution within and exports of elements from the soil profile by leaching processes (Kalbitz et al., 2000). It also plays an important role for the distribution of PyC between environmental compartments; dissolved PyC has been reported from soil leachates, rivers and oceans (Mitra et al., 2002; Hockaday et al., 2006; Hockaday et al., 2007; Dittmar & Paeng, 2009; Ziolkowski & Druffel, 2010). Increasing functionalization upon ageing, e.g. oxidation could lead to an increased solubility (Lehmann et al., 2005). But it is not clear how much of PyC present in charcoals gets solubilized and if this fraction can increase as charcoals become more functionalized upon ageing. Here solubility of laboratory produced standard charcoal was compared to the solubility of aged charcoal, which was exposed to weathering for more than 10 years.

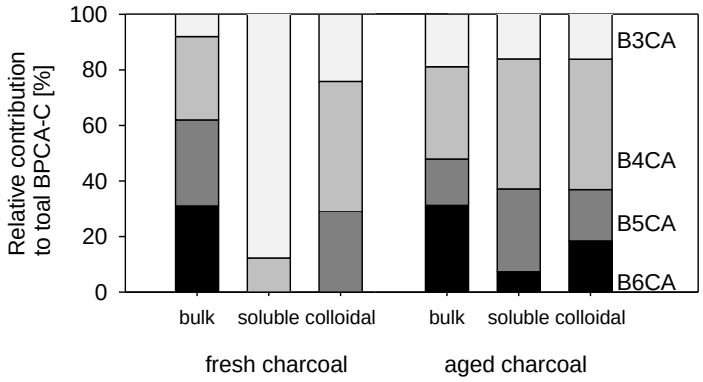

Figure 9: Relative contributions of BPCA marker molecules (B3CA: hemimellitic and trimellitic acid; B4CA: prehnitic, mellophanic and pyromellitic acid, B5CA: pentacarboxylic acid; B6CA: mellitic acid) in bulk, soluble and colloidal fractions of fresh and aged charcoals. Compared to soluble PyC from fresh charcoal, the soluble fraction from aged charcoal shows higher contribution of B6CA molecular marker, indicating the presence of more condensed aromatic structures.

For both tested charcoals, solubilization of PyC was very low, soluble and colloidal fraction representing < 0.3% of total charcoal-C in all cases. However, the soluble fraction increased strongly upon ageing, 40-55 times more C was released from the aged charcoal compared to charcoal from the laboratory. For fresh charcoal the soluble and colloidal fraction only yields B3CA, B4CA and B5CA, indicating maximum cluster sizes of 4 condensed rings (Figure 9). For soluble and colloidal fractions obtained from the aged charcoal, the presence of B6CA in the digest indicates minimum cluster sizes of 5 or more aromatic rings. It appears that upon ageing of PyC even the higher condensed aromatic units get more polar by substitution with functional groups, and thus more water soluble (Dittmar & Koch, 2006). Consequently, with increasing residence time in soil, higher condensed aromatic units of 5-7 rings would become water soluble.

The relative contribution of B6CA-C in the soluble fraction of aged charcoal is 7.3% of total BPCA-C. Similar contributions of B6CA were observed in samples taken in the Gulf of Mexico (Dittmar, 2008) and in a region where the Amazon river enters the Atlantic Ocean (Ziolkowski & Druffel, 2010). This could be an indication that once solubilized, PyC does not get much transformed on its way from the soil profile to rivers and to the coastal ocean waters. In contrast, open ocean samples taken in the SE

Atlantic and N Central Pacific did not yield any B6CA , which could be indicative for photochemical oxidation in ocean surface waters, leading to breakdown of PyC into smaller subunits (Ziolkowski & Druffel, 2010).

4.5 Tropical weathering over a century does not change the molecular marker signature of charcoal

Degradation of PyC in soil has been reported in a steppe soil (Hammes et al., 2008b), savanna soil (Bird et al., 1999) and tropical soil (Nguyen et al., 2008). The PyC degradation in soils is mainly driven by microbial activities and via co-metabolic pathways, as suggested by laboratory studies (Hamer et al., 2004; Kuzyakov et al., 2009). Here the BPCA method was applied to a chronosequence of soils from western Kenya, with increasing time (0-100 years) since the last PyC input in order to follow chemical changes in PyC under intense weathering in a tropical climate. In the soil chronosequence samples indications for PyC degradation processes come from an increasing functionalization of surfaces observed on macroscopic charcoal pieces by spectroscopic methods (Nguyen et al., 2008). A stabilization of PyC can result from organic-mineral interactions, which leads to a protection of PyC from microbial degradation (Brodowski et al., 2005a; Knicker, 2011). For the chronosequence samples an increasing presence of Al, Fe and Si on the surface of macroscopic charcoal pieces indicated such interactions also occurred here (Nguyen et al., 2008). However, as yet it is not clear if the finely distributed bulk (micro-)charcoal is protected against microbial degradation by such mechanisms or if its quality changes with increasing residence time in soil due to degradation.

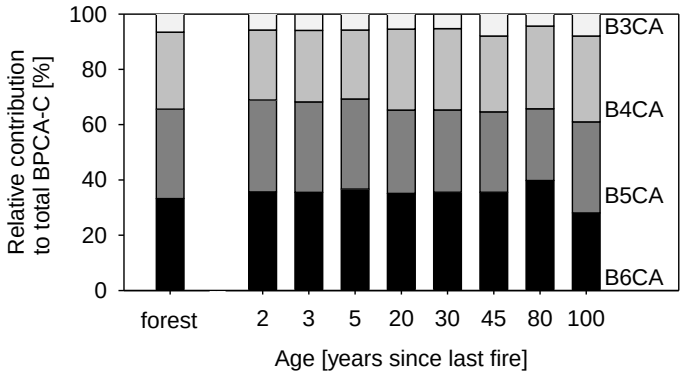

Figure 10: Relative contributions of BPCA marker molecules (B3CA: hemimellitic and trimellitic acid; B4CA: prehnitic, mellophanic and pyromellitic acid, B5CA: pentacarboxylic acid; B6CA: mellitic acid) in soil chronosequence samples with increasing time of conversion from forest to agricultural land by slash-and-burn. The "forest" sample represented the pre-existing PyC stocks in the forest soil. B6CA, a measure for the degree of condensation in PyC, did not change over the observation period (n=3; 45 years sample n=1).

The main finding of the study was that there were no changes in the relative distribution of BPCA over the whole period of 100 years in bulk PyC (Figure 10). This indicates that even under accelerated element cycling in a tropical agro-ecosystem the condensed aromatic structure of PyC could be resistant to degradation. These findings are in contrast to results from two profiles in a Russian steppe soil (Hammes et al., 2008b), where over 100 years degradation of PyC has been found to be reflected in changes in the relative BPCA contribution of individual molecular markers: A relative

enrichment of B6CA indicated a preferential accumulation of more oxidation resistant, highly condensed PyC materials. In contrast, another study followed changes in PyC under two different management practices (bare fallow versus native steppe vegetation) over 60 years, and observed no changes in PyC stocks or BPCA relative contribution, whereas TOC was clearly depleted by 33% in the bare fallow plots (Vasilyeva et al., 2011).

In section 4.4 it was shown that aged charcoal also releases higher condensed aromatic units into solution (indicated by the presence of B6CA), which would mean that the process of solubilization and leaching does not necessarily lead to an enrichment of highly condensed aromatic molecules in the insoluble bulk PyC over time. This would fit with the observation made here that no changes became evident in bulk PyC over time. Also, potential losses from the topsoil in this study could be explained by physical (particulate) export via runoff, rather than by (bio-)chemical degradation, which probably would be reflected in a change of the BPCA pattern.

Besides a relative enrichment of highly condensed PyC fractions during degradation, it is also possible that ageing of PyC leads to a depolymerization of the highly condensed aromatic backbone of PyC and breaks down bigger condensed units into smaller subunits (Kaal et al., 2009). This would result in a change of the BPCA pattern towards higher contributions of less carboxylated marker molecules (B3CA, B4CA, B5CA). In an incubation study, which was conducted over 2 years at 20 °C, the BPCA pattern of incubated charcoals changed only slightly, showing minimal increasing contribution of B3CA on the expense of B5CA (Brodowski, 2005). However, the influence of degradation processes and leaching on the relative contribution of BPCA yielded from charcoals and the mechanisms involved remain obscure and clearly require further attention.

5 Conclusions

In this work it could be shown that benzene polycarboxylic acids (BPCAs) are a valuable tool to determine pyrogenic carbon PyC in the environment. More specifically, it was shown that:

1. A modified method using liquid chromatography for BPCA analysis reduces efforts in sample preparation and analytical uncertainty.
Through the modification of the method towards the use of liquid instead of gas chromatography, procedural errors could be reduced and analyses became less time consuming. Furthermore, yields of BPCA from standardized charcoal test materials could be increased, due to smaller sample losses during preparation.

2. The yield of B6CA in charcoals is indicative for the heat treatment temperature.
For the first time, the quantitative and qualitative information provided by the BPCA method was calibrated using a series of reference charcoals of known heat treatment temperature and biomass source. One of the molecular markers, B6CA, is indicative of highly condensed aromatic molecules. The yield of this marker molecule consistently increases with heat treatment temperature with most pronounced increases at temperatures from 500-800 °C, which indicates the growth of graphene-like structures at these temperatures. Based on the relative contribution of the B6CA marker molecule it is possible to distinguish low temperature charcoals (< 500 °C) from high temperature charcoals (> 500 °C). The B6CA measure can be used to estimate the degree of condensation in other heat treated materials, such as charcoals produced in traditional kilns or so-called biochars produced for agricultural use.

3. Molecular markers measure a representative subfraction of PyC.
Further, it was demonstrated that the method measures a relevant subfraction of PyC in charcoals over a broad temperature range, covering temperatures also common in wildfires. Slightly charred material (heat treatment temperatures < 300 °C) is not fully detected by the method, because it has only a minor contribution of condensed aromatic structures. At highest heat treatment temperatures (800-1000 °C) a slight decrease in total BPCA yields was observed, indicating that the highly condensed aromatic structures are less effectively converted to BPCA molecular markers.

4. Ageing of charcoal promotes solubility of more condensed aromatic units.
Ageing of charcoals results in an increase of quantity released into water and changes the quality of the soluble fraction towards more condensed aromatic units. These results show that export of PyC via leaching can be an important process to explain long-term losses of PyC from the soil profile, especially in regions with high precipitation.

5. Charcoal is more resistant to intense tropical weathering than expected.
No indications were found for an accumulation of highly condensed aromatic structures in bulk PyC of a tropical agricultural soil. The absence of detectable changes indicates that finely distributed PyC might be highly resistant against degradation even under forced tropical conditions, possibly due to protection through PyC-mineral interactions.

6 Research perspectives

6.1 Global PyC budget

The global PyC budget is still associated with large uncertainties. What are the next necessary steps to set the basis for the calculation of a global PyC budget? The aim is one common, LC-based BPCA method for all environmental compartments. This would make BPCA analyses less time consuming, while analytical uncertainties could be reduced. An application to aerosol samples might become possible if the required sample amounts can be further reduced. These are important prerequisites for a broader application of the BPCA method to all environmental compartments, in order to close currently existing gaps in the global PyC budget. The BPCA method offers the chance to get comparable results for PyC quantity and quality from the quantitative most important reservoirs in the global PyC cycle: soils, sediments and waters.

6.2 Future applications of PyC molecular markers

In terms of quality information it would be important to know, how molecular marker distributions yielded from charcoals produced in wildfires compare to the distribution observed in the thermosequence charcoals. Materials produced in prescribed burns with monitored fire temperatures are especially suitable for such comparison.
The effect of microbial and chemical degradation of charcoals on the molecular properties of PyC and the resulting changes in the BPCA pattern might be investigated in laboratory incubation and field studies, which would lead to a better understanding of degradation processes of PyC and how these are related to changing BPCA patterns.

6.3 Method standardization

Any modification of the BPCA method must be tested on reference materials for e.g. charcoal, soil and sediment samples (Hammes et al., 2007) in order to assure comparability with previous results. The reproducibility of results can be monitored using in-house standard materials, also using the reference materials from the ring trial study (Hammes et al., 2007), such as Chernozem soil or wood and grass charcoals.
Laboratory produced charcoal thermosequences can serve as standardized test materials covering a broad range of PyC properties. Existing and newly developed methods for PyC characterization should be applied to these thermosequences in order to systematically compare results and better assess the performance of each method over the combustion continuum.

6.4 Method development

Compound specific radiocarbon analysis (CSRA) is a powerful tool to trace the origin and fate of specific molecules in the environment. The BPCA molecular marker method enables CSRA for PyC, because the specific PyC molecular markers can be isolated and collected via preparative chromatography and subsequently analyzed for their ^{14}C content. As yet, CSRA of PyC molecular markers using preparative GC requires great efforts for preparation and measurement and can only be applied to a very limited number of samples due to the high costs involved in this analysis (Ziolkowski & Druffel, 2010). Further, GC separation involves derivatization with foreign carbon with a different ^{14}C signa-

ture, which thus requires correction of the measured ^{14}C content (Ziolkowski & Druffel, 2009b). ^{14}C analysis of BPCA using preparative HPLC for purification is frustrated by the fact that an organic modifier is required in the mobile phase to achieve good separation. Here, further method development is clearly required, in order to come to CSRA of PyC with a minimum of sample handling and sources of error (e.g. through external C addition), which would be an important prerequisite for a broader application of CSRA for pyrogenic carbon.

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PART B – Manuscripts

Manuscript 1

The benzene polycarboxylic acid (BPCA) pattern of wood pyrolized between 200 °C and 1000 °C

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The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 °C and 1000 °C

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ABSTRACT

Environmental charcoals represent a poorly defined part of the black carbon (BC) combustion continuum and may differ widely in their chemical and physical properties, depending on combustion conditions and source material. The benzene polycarboxylic acid (BPCA) molecular marker method is well established to quantify the BC component in charcoal, soil and sediment, although observed variations between labs could stem from subtle differences in methods. The objectives of this study were to identify and improve potential sources of analytical uncertainty. The improved method was then used to qualitatively characterize wood charred at 200–1000 °C. One significant improvement of the BPCA method was to replace citric acid with phthalic acid as an internal standard, which is more stable in acidic solution and more similar to the target compounds. Also, including a soil reference material as a quality control in each analysis proved to be a robust tool to detect for variations in reproducibility. For the thermosequence, elemental O/C and H/C ratios typically decreased with temperature to $\leqslant$0.03 at 1000 °C, whereas BPCA concentrations peaked at 700 °C. With temperature B6CA proportions increased consistently (6–98%), except for a plateau at 250–500 °C. Thus, relative contributions of B6CA reflected the pyrolysis temperature and probably also the degree of condensation of the charcoals we investigated. Future work will show if our results can be directly related to charcoal produced under oxygen limited conditions, including charcoal formed at wildfires or so called biochar for agricultural use.

1. Introduction

Charcoals in the environment form a part of the black carbon (BC) combustion continuum (Masiello, 2004). Charcoals differ in their chemical and physical properties depending on several formation factors, including feedstock properties, the gas environment, the thermal ramp rate and final temperature of the carbonization process (Antal and Gronli, 2003; Brown et al., 2006). With increasing formation temperature, the pH, C content, degree of condensation, specific surface area and sorption capacity for organic pollutants of charcoals typically increase (Shafizadeh and Sekiguchi, 1983; Brown et al., 2006; Gundale and DeLuca, 2006; Chen et al., 2008), while the degradability of charcoals decreases (Bruun et al., 2008; Nguyen and Lehmann, 2009; Nguyen et al., 2010; Zimmerman, 2010). Thus, properties of charcoals produced at different temperatures may cover the whole spectrum of the BC continuum, i.e. from slightly charred biomass produced at temperatures as low as 200 °C to highly aromatic charcoals at very high temperatures. Many methods used to quantify charcoal do not reflect the degree of aromatic condensation of the charcoal, e.g. when determined as oxidation residues (Wolbach and Anders, 1989; Gustafsson et al., 1997, 2001; Simpson and Hatcher, 2004a,b). Additional qualitative information about the charcoal can be obtained from spectroscopic (Smernik et al., 2006; McBeath and Smernik, 2009; Keiluweit et al., 2010) and molecular marker methods (Hammes et al., 2007; Kaal and Rumpel, 2009).

Presently, there are several published molecular marker methods to measure charcoal, including analytical pyrolysis (Kaal et al., 2009), a method identifying levoglucosan (Simoneit et al., 1999) and the benzenepolycarboxylic acid (BPCA) method (Glaser et al., 1998; Brodowski et al., 2005). Analytical pyrolysis and the levoglucosan method are well suited to detect and characterize charcoals (Kuo et al., 2008; Kaal et al., 2009), but only the BPCA method yields quantitative information and additionally qualitative information about the degree of condensation of BC (Glaser et al., 1998; Brodowski et al., 2005). The BPCA method allows to assess the relative contributions of individual molecular markers, which reflect the size of the aromatic clusters. Although several authors have taken advantage of this unique feature of the BPCA

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method (Glaser et al., 1998; Brodowski et al., 2005; Dittmar, 2008; Hammes et al., 2008b; Ziolkowski and Druffel, 2009) a systematic testing of how the BPCA pattern reflects charring temperature is still lacking (Hammes et al., 2007).

Testing the temperature dependence of the BPCA pattern, however, would require a highly reproducible analytical method covering the whole combustion continuum. The BPCA method has been used in many publications over the past decade (e.g. Rodionov et al., 2006; Brodowski et al., 2007; Hammes et al., 2008b), and Hammes et al. (2007) pointed out that the BPCA method is well suited for BC analysis in soils. In the "ring trial" study of Hammes et al. (2007) in which 17 laboratories participated, seven methods for BC analysis were tested on 12 reference materials. It became clear from the ring trial that the BPCA method might underestimate BC in highly condensed aromatic molecules, such as soot. On the other hand, it was shown that there is a positive bias from non-BC materials like shales and coals. These potential limitations must be considered when interpreting results obtained by the BPCA method for samples that contain such forms of carbon.

Further, interlaboratory reproducibility should be improved. In the ring trial, BC concentrations varied by factors of two or more when analyzed with the BPCA method in different laboratories. The essential first step, breaking BC molecules into the BPCA, i.e. the nitric acid oxidation at 170 °C, proved to be robust and reproducible (Glaser et al., 1998; Dittmar, 2008). However, the ring trial revealed that the many cleaning, transfer and derivatization steps with seemingly minor handling differences between laboratories could have resulted in disparate results.

More specifically, Hammes et al. (2007) identified several potential sources of error during BPCA analysis. First, internal standards (IS) are used to detect possible losses during sample cleaning over the resin, freeze drying and subsequent transferring the samples into vials. In BPCA analysis, citric acid has been used as IS, despite its known instability under strongly acidic conditions (Brodowski et al., 2005) and despite the fact that its chemical structure is not very similar to the BPCA. Phthalic acid is structurally more similar to the target compounds and so might be less susceptible to deterioration and could serve as a better internal standard. Second, extracted soil and charcoal samples often need to be stored before further processing. BPCA themselves are known to be very stable in solution, but they might interact even with traces of polyvalent metal ions (Glaser et al., 1998), and thus affect results. But the stability of BPCA extracts from soil and charcoal samples in strongly acidic solution has not yet been tested systematically. Third, Glaser et al. (1998) observed that, once derivatized, standards and soil extracts remained stable for up to one week when stored dry and our observation was that even traces of humidity in the vial may affect results.

The first objective of this study is to identify possible sources of error during sample handling and preparation and to propose more robust alternative steps in Section 2. The improved method is then used for characterization of a charcoal thermosequence. In Section 3 we focus on the following question: Does charcoal pyrolyzed at temperatures ranging from 200 to 1000 °C (charcoal thermosequence) produce a systematic and consistent BPCA pattern?

2. Experimental

2.1. Materials

2.1.1. Reference soil material

To test for reproducibility of the BPCA method in soils we selected a Chernozem soil (Schmidt et al., 1999), which has already been used in a multi-lab, multi-method comparison study (Hammes et al., 2007). The Chernozem soil was sampled at 20–60 cm depth in the Hildesheim-Braunschweig region (Harsum, Germany) with 19 wt% clay content and 20.1 g OC/kg.

2.1.2. Charcoal thermosequence

The thermosequence includes the reference wood charcoal charred at 450 °C used in the ring trial study (Hammes et al., 2007), which was characterized in Hammes et al. (2006, 2008a). All other charcoals in the thermosequence were derived from the same source of biomass, cut into small wood chips (2–8 mm) and produced according to the protocol described in Hammes et al. (2006). Debarked and cut chestnut wood (*Castanea sativa*) was charred in a pyrolysis furnace (Carbolite CTF 16/75, Sheffield, UK) under a N_2 atmosphere (flow 13 l/h). The temperature was raised from room temperature to 200 °C at a rate of 300 °C/h and then to 250–1000 °C at a rate of 50 °C/h. The respective maximum temperature (200–1000 °C) was held constant for 5 h to assure complete charring. In total, we produced a thermosequence of 12 charcoals (Table 1). The charcoal was collected after cooling (8 h) and stored in a cool, dry place in glass bottles with screw caps (Schott, Mainz, Germany). Samples were weighed before and after charring to determine mass loss.

2.2. Methods

2.2.1. Black carbon analysis using the BPCA method

We used the BPCA method as described by Glaser et al. (1998) with the modifications suggested by Brodowski et al. (2005). In short, the method involves a pretreatment step with 4 M trifluoroacetic acid (TFA) to remove Fe and Al, followed by the conversion of BC to BPCA by nitric acid oxidation at 170 °C for 8 h. The extract is then percolated over a cation exchange resin to remove remaining polyvalent cations. Subsequently, the samples are freeze dried and finally converted to trimethylsilyl derivatives for GC-FID (gas chromatograph equipped with flame ionization detector) analysis. All analyses were done in triplicate. For the BPCA analysis, about 500 mg of soil sample or 2–100 mg of charcoal sample (1.9–50.3 mg OC, depending on the expected BC content in order to avoid GC column overload for high BC contents) was used.

For the measurements of the charcoals with expected high BC contents (300–1000 °C, Fig. 5), we doubled the amount of B5CA and B6CA standards (200 μg instead of 100 μg in 100 μl) to adjust the standard series to the higher amounts of B5CA and B6CA in the samples.

Total BPCA amounts were calculated as the sum of the seven marker molecules hemimellitic, trimellitic, pyromellitic, prehnitic, mellophanic, pentacarboxylic and mellitic acid. All BPCA data reported here was calculated without using the conversion factor 2.27 (Glaser et al., 1998). For a detailed discussion see Section 3.1.

Further modifications and additional testing of the method are described in Sections 2.2.2–2.2.5, addressing the following questions:

- Can phthalic acid replace citric acid as internal standard, i.e. is it less susceptible to degradation under acidic conditions?
- How long can samples be stored in the acidic extract after HNO_3 oxidation without changes in quantity or quality?
- How long can derivatized standards and soil extracts be stored without changes in quantity or quality?
- How reproducible are BPCA measurements using a soil reference material?

2.2.2. Acid stability tests of external and internal standards (citric and phthalic acid)

When adding the internal standard during sample preparation, the pH of the extract solution typically ranged from 0.8 to 1.9. We tested the stability of the BPCA in standard solution and of the

Table 1
Properties of chestnut wood (*Castanea sativa*) both fresh and pyrolyzed at temperatures from 200 to 1000 °C under N_2 stream.

Charring temperatures (°C)	Wood[a]	200	250	275	300	350	400	450[a]	500	600	700	800	900	1000
C (g/kg)	458	503	544	642	695	734	781	682	871	938	951	960	965	963
H/C	1.6	1.32	1.16	0.79	0.69	0.52	0.46	0.7	0.36	0.24	0.14	0.08	0.04	0.03
O/C	0.8	0.66	0.55	0.37	0.28	0.24	0.18	0.3	0.08	0.03	0.03	0.02	0.02	0.02
Mass loss of initial (%)	–	12.0	29.0	47.8	55.0	59.0	69.0	60.0	69.5	74.5	72.5	73.0	75.0	73.0

[a] All data for wood and 450 °C charcoal from Hammes et al. (2006).

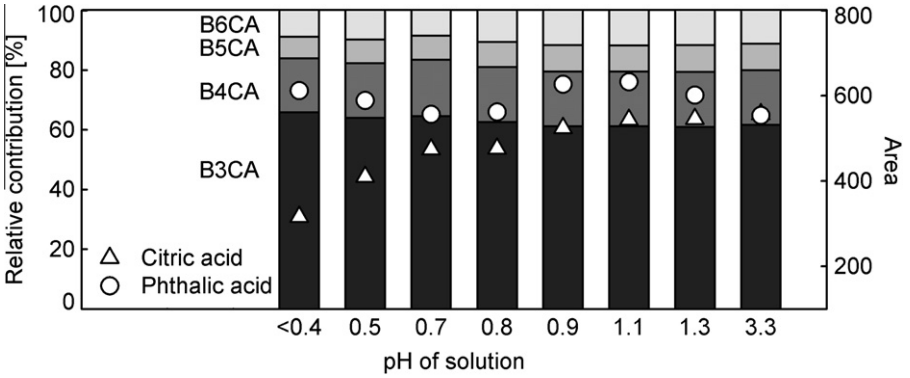

Fig. 1. Detection of BPCA and two internal standards at different pH. Relative contribution of individual BPCA (left scale) and areas of citric and phthalic acid (right scale) as obtained by GC-FID measurements of standard solutions. Below pH 1 the detected area of citric acid decreases, whereas phthalic acid and BPCA are not affected by low pH.

presently used internal standard citric acid compared to phthalic acid (Fig. 1). External standards (hemimellitic, trimellitic, pyromellitic, pentacarboxylic and mellitic acid) were prepared by dissolving 50 mg of each compound in 100 ml of deionized water in volumetric flasks. About 100 mg of internal standard citric acid were introduced in 100 ml volumetric flasks and filled up with deionized water. The same was done with the alternative internal standard phthalic acid. To test for the stability of both compounds with decreasing pH, 100 µl of each standard solution was exposed to increasing concentrations of HNO_3 and the pH of the solutions was measured with an IQ240 glass electrode (IQ Scientific Instruments). After that the solutions were freeze dried and prepared for GC-FID analysis.

At pH < 1.0, typically encountered during sample preparation, BPCA and phthalic acid concentrations did not change, whereas citric acid concentrations decreased (Fig. 1). These results confirm that citric acid is not an ideal internal standard for BPCA analysis (Brodowski et al., 2005). Phthalic acid, on the other hand is not only stable under acidic conditions, it also does not co-elute with any of the BPCAs (Fig. 2a), and its chemical structure is more similar to the target compounds. When adding both internal standards to a set of samples for cross checking, we recovered more than 90% of the phthalic acid. In contrast, we only recovered 69% of the citric acid, which proves the instability of citric acid in the acidic sample solution.

It could be possible, however, that phthalic acid is produced during charcoal oxidation, or that plasticizers in lab plastic contaminate samples with phthalic acid. To check for the presence of artifact phthalic acid, we prepared samples as described above without adding phthalic acid (Fig. 2b) and found negligible contributions of phthalic acid after oxidizing Chernozem soil samples with HNO_3 (<0.6% of total quantified area). Thus, we strongly recommend replacing citric acid by phthalic acid as an internal standard.

2.2.3. Stability of BPCA extracts from soils over time

The BPCA in the non-purified, acidic soil extracts could interact with other components (e.g. polyvalent cations; Glaser et al., 1998)

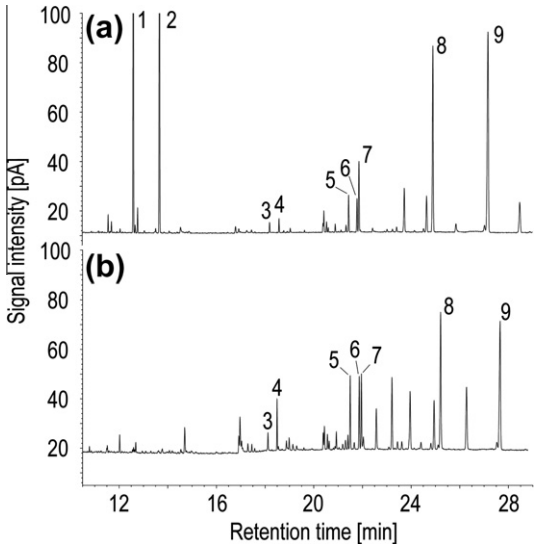

Fig. 2. Gas chromatograms of benzene polycarboxylic acids (BPCA) obtained from two independent nitric acid extractions from reference soil (Chernozem). (a) Phthalic and citric acid were added. Phthalic acid does not co-elute with other BPCA. (b) No phthalic acid was added to test for contamination. There is negligible contribution of phthalic acid (<0.6% of total quantified area). (1) Phthalic acid (alternative internal standard); (2) citric acid (internal standard); (3) hemimellitic acid; (4) trimellitic acid; (5) pyromellitic acid; (6) mellophanic acid; (7) prehnitic acid; (8) benzene pentacarboxylic acid and (9) mellitic acid.

when stored over longer time (days to weeks). Even after dilution (1:5 with deionized water), the HNO_3 extract had a very low pH of 1.8 and after several days or weeks BPCA might partly deteriorate, or might interact with the substances in the solution. To test the BPCA stability at intermediate timescales (up to 1 month) in the non-purified soil extracts after HNO_3 oxidation (Section 2.2.1), sample extracts were stored for 3, 8 and 30 days in the diluted acid before further sample processing. Total BPCA carbon and relative contributions of individual BPCA extracted from the Chernozem reference soil did not change during 30 days of storage at room

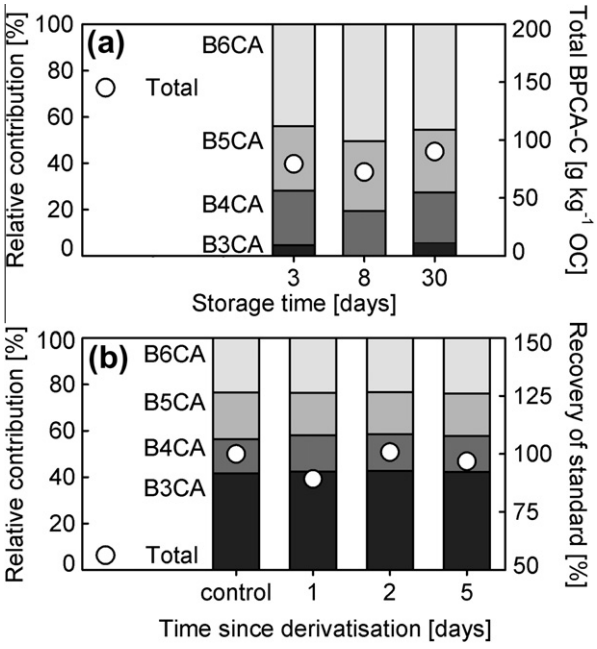

Fig. 3. (a) Stability of soil BPCA extract stored in acidic solution (pH 1.8) for 3, 8 and 30 days at room temperature. Relative contribution of individual BPCA (left scale) and total BPCA-C content (right scale) in Chernozem soil (SE for analytical replicates, $n = 3$). No changes in quality and quantity were observed, which shows the stability of underivatized BPCA at low pH. (b) Stability of BPCA standard added to soil extract. Measured after 1, 2 and 5 days after derivatization. Pure standard mixture of BPCA was used as a control. Relative contribution of individual BPCA (left scale) and recovery of standard mixture (right scale, control = 100%). (SE for analytical replicates, $n = 3$). No changes in quality and quantity of BPCA were observed in 5 days.

temperature under acidic conditions (Fig. 3a). Thus, after HNO_3 oxidation we can store extracts for up to a month before further sample processing without influencing the results.

2.2.4. Derivatization of BPCA standards and their long term stability

Brodowski et al. (2005) found that complete derivatization of BPCA with BSTFA + TMCS 99:1 requires at least 24 h before injection into GC. The long term maximum storage time after derivatization, however, has not yet been tested (Brodowski et al., 2005). We derivatized BPCA standards and quantified them 12 times over a period of 254 days. We could not detect any major changes in quantity or quality of the BPCA (data not shown).

In soil extracts, however, other than in pure standard solutions, derivatized BPCA probably could interfere with substances that survived the digestion and purification treatments of the samples material (e.g. traces of polyvalent cations). To test this, we took two sub-samples from a Chernozem nitric acid extract. Standards containing 80 μg of each BPCA were added to one of the two sub-samples before derivatization. Samples were then derivatized and analyzed by GC-FID. For comparison, we subtracted the areas obtained for soil sub-samples from the areas obtained for mixed soil/standard sub-samples. The pure standard mixture was used as a control. Total BPCA-C concentrations were slightly lower for measurements with standard addition (Fig. 3b), but the BPCA pattern did not differ. We recovered 89.2, 100.8 and 96.8% of the added standards from soil extracts compared to pure standard solution after 1, 2 and 5 days, respectively (Fig. 3b). Thus, derivatized BPCA seem to interfere with soil extracts, although differences were only small and did not increase with time.

2.2.5. Quantitative and qualitative reproducibility of the BPCA method tested with a reference soil

In total, we performed 34 independent triplicate analyses of the reference soil and selected those 16 for further statistical analysis with standard errors <3.5 (Fig. 4).

From the repeated analysis of the reference soil we conclude that the following criteria should be considered when interpreting results obtained by the BPCA method: (1) The recovery of the internal standard reveals losses during sample processing and should at least reach 70%. (2) The slope of the external mellitic acid standard series obtained by a linear regression is a good measure for the success of derivatization. Slopes below 0.7 indicate that a considerable part of mellitic acid standard has not been successfully transformed to trimethylsilyl derivatives.

Those runs that failed to meet criteria 1 and/or 2, were excluded from further analysis. The repeated BPCA extractions of the soil reference material yielded a mean of 66.2 g BPCA-C/kg OC (Fig. 4). We applied a normal distribution to the reference soil data set (standard deviation SD = 15.2, standard error SE = 2.3, $n = 44$). We concluded that the BPCA-C content of the reference soil probably lies between 51.1 and 81.4 g/kg OC (as defined by the mean ±1SD). BPCA analyses that yield higher or lower contents could point to losses or inaccuracies during sample processing.

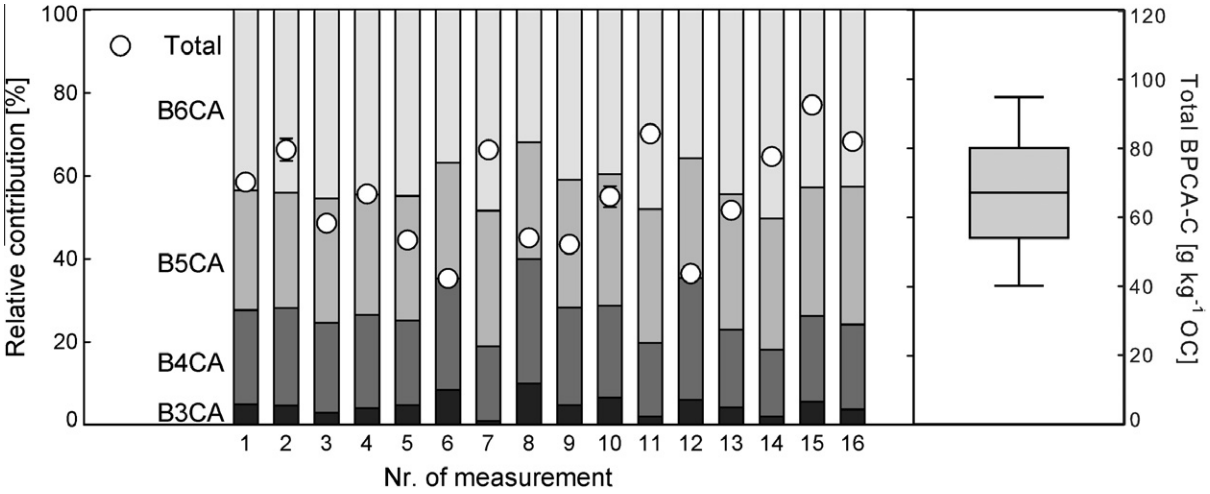

Fig. 4. BPCA-C content of Chernozem reference soil (white circles, right scale) as obtained by 16 independent measurements (SE for analytical replicates, $n = 3$). Relative contribution of individual BPCA (left scale) to total BPCA-C content is shown as stacked bars. The box plot on the right shows the median (black line, 67.1 g BPCA-C/kg OC) and the interquartile range of the dataset (grey box, 53.6–80.0 g BPCA-C/kg OC), the whiskers show minimum and maximum values.

There was still some considerable scatter in the data even with the modifications introduced, with contents between 40.2 and 94.8 g BPCA-C/kg OC (Fig. 4). For quality control, samples should be analyzed together with a known reference material. As always, the reference material used should have properties similar to the analyzed material (e.g. soil or marine sediment) and could include those materials already used in the ring trial (Hammes et al., 2007).

Runs which lay outside the above defined range should be considered to be repeated, either starting with a new HNO_3 extraction or with a new clean up of the stored extract (see Section 2.2.3). Results obtained from the reference material could then be used to correct for possible variations in analysis. In terms of the quality of the BC detected in the reference Chernozem soil, B6CA contribution varied between 32–50%, but most values were between 40–45% (Fig. 4). B3CA always made up the lowest fraction of all BPCA with contributions between 1 and 10% of total BPCA-C contents.

2.2.6. Other measurements

Elemental analysis (CHO) of the charcoals was carried out on a LECO CHN-900 and a LECO RO-478 (Mönchengladbach, Germany) in two laboratory replicates.

2.2.7. Statistics

A normal distribution of the data was tested with a Kolmogorov–Smirnov test ($p < 0.05$). All statistical comparisons were conducted with SPSS version 17.0.

3. Results and discussion

3.1. Consistency and plausibility of BPCA data tested on a charcoal thermosequence

The term BC is operationally defined and does not describe a substance with a defined chemical structure (Goldberg, 1985; Masiello, 2004). BC comprises all organic material that was subjected to various degrees of heating and different analytical methods capture a different part of the combustion continuum (Hammes et al., 2007). Consequently, no "true" value for the BC content of a material exists, which we could test. Applying a thermosequence of charcoals allows us to test the BPCA method for reproducibility, consistency and plausibility, but not for accuracy per se.

Not all carbon in charcoals is detected as BPCA-C. A conversion factor can be used to account for the carbon losses during sample processing, e.g. during HNO_3 oxidation in form of CO_2 and to convert BPCA-C contents into "black carbon" contents. This is done in order to obtain results that give more realistic values for BC contents in soils. The conversion factor of 2.27 is based on the mean BPCA-C contents of 441.2 g/kg OC obtained for activated and barbecue charcoal (Glaser et al., 1998). In our study we found maximum BPCA-C contents of 155.3 g/kg OC in the charcoal thermosequence. Brodowski et al. (2005) found maximum conversion factor for charcoals exceeding 4.5, which would better fit with our results but on the other hand would lead to an overestimation when applied to soils (Brodowski et al., 2005).

Also it becomes clear from our study yielding between 3.3 and 155.3 g BPCA-C/kg OC for the thermosequence charcoals that the application of a single conversion factor for different types of charcoal will always be incorrect in all but one special case, i.e. the test substance, and every charcoal which happens to be identical in chemical structure. Thus, the data presented here strongly supports the statement that any correction factor must be wrong and introduces an additional error to BC quantification. In consequence, we recommend to not use any correction factor at all, but to report BPCA-C content in soils and charcoals as it is.

3.2. Elemental composition

With increasing temperature, wood showed a consistent decrease of mass, with no major changes >600 °C (Table 1). Elemental concentrations of C, H and O typically are used to represent the composition of organic matter. With increasing charring temperature, H/C and O/C ratios decreased, indicating an increasing degree of thermal alteration of the initial biomass, similar to observations of others (Baldock and Smernik, 2002; Antal and Gronli, 2003; Hammes et al., 2006; Keiluweit et al., 2010; Zimmerman, 2010). The observed trends are in good agreement with the change in elemental composition with increasing temperature of other heat treated biomass like cellulose (Shafizadeh and Sekiguchi, 1983; Nishimiya et al., 1998), wood (Nishimiya et al., 1998; Baldock and Smernik, 2002; Brown et al., 2006; Keiluweit et al., 2010), grass (Keiluweit et al., 2010) and peas (Braadbaart et al., 2004). At the same temperature, absolute values for H/C and O/C ratios can vary significantly, depending on the source material and pyrolysis conditions. Longer exposure time to the final pyrolysis temperature, e.g. 5 min in Shafizadeh and Sekiguchi (1983) or 1 h in Keiluweit et al. (2010), led to lower H/C ratios at the same temperature compared to H/C ratios of the thermosequence charcoals (5 h pyrolysis), which indicates ongoing pyrolysis reactions at shorter time.

3.2.1. Black carbon quantity

We quantified total BC concentrations of the samples as BPCA-C (Fig. 5). The BPCA-C content measured for the reference charcoal (wood 450 °C) in our study fits with the results obtained in the ring trial study (Hammes et al., 2007, auxiliary material Text S2) for two of three participating laboratories (161.9, 146.1 and 47.1 g BPCA-C/kg OC, this study: 137.2 g BPCA-C/kg OC, all data without using a conversion factor).

BPCA-C concentrations of the charcoals increased with charring temperature (200–700 °C), while H/C decrease (Table 1), pointing to formation of structures containing unsaturated C such as aromatic rings. Elemental data (Shafizadeh and Sekiguchi, 1983; Antal and Gronli, 2003) and spectroscopic techniques (Knicker et al., 1996; Baldock and Smernik, 2002; Czimczik et al., 2002) suggest increasing contents of aromatic C in woody biomass subjected to increasing levels of thermal alteration. These observations show a consistent trend despite very different heat treatment conditions, e.g. availability of oxygen and exposure time in the studies.

At higher temperatures (800–1000 °C), however, BPCA-C content decreased again, although small O/C ($\leqslant$0.02) and H/C ($\leqslant$0.08) ratios suggested a high degree of condensation. One possible explanation for the apparent discrepancy between elemental and molecular data is an incomplete HNO_3 digestion, which does not convert the very stable charcoal to BPCA. This is in accordance with the findings of the ring trial (Hammes et al., 2007), where it was shown that highly condensed structures such as soot partly escape the analytical window of the BPCA method.

3.2.2. Black carbon quality

The contributions of individual BPCA molecular markers can be used to infer the size of the aromatic clusters (e.g. Hammes et al., 2008b). Aromatic rings at the outer margin of a black carbon molecule are more likely to produce B3CA, B4CA and B5CA, whereas B6CA can only originate from the core of the black carbon molecule. In other words, larger proportions of B6CA would reflect (on average) higher degrees of condensation of a sample.

The charcoals analyzed in this study showed that with increasing temperature relative contributions of B6CA consistently increased (Fig. 5). Low temperature charcoals (final temperature 200 °C) produced very little (<10%) B6CA. Charcoals produced at intermediate temperatures (250–500 °C) produce more B6CA (10–40%) and in the high temperature charcoals (600–1000 °C)

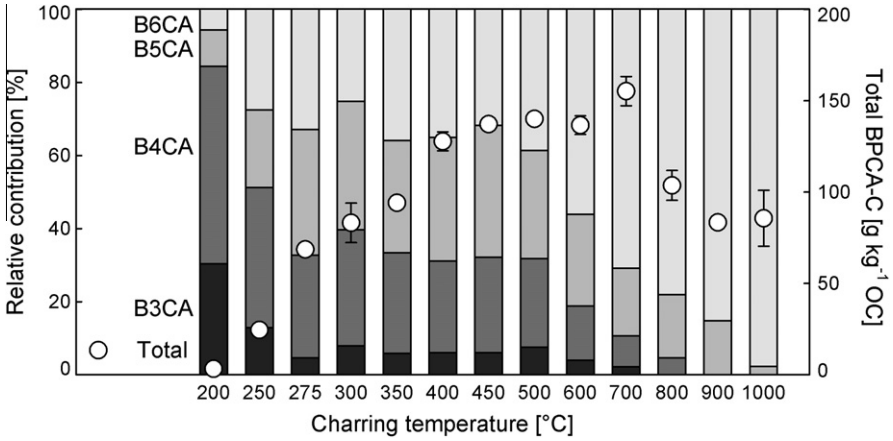

Fig. 5. BPCA-C content of chestnut wood charcoals (white circles, right scale) produced at increasing charring temperatures (SE of analytical replicates, $n = 3$). Relative contribution of individual BPCA to total BPCA-C content is shown as stacked bars (left scale). BPCA-C contents of wood charcoals consistently increased for charring temperatures from 200–400 °C and peak at 700 °C. For charring temperatures >700 °C BPCA-C contents decreased. The relative contributions of the B6CA molecular marker strongly increased with charring temperature.

B6CA contributed most to the total BPCA content (>40%). A strong increase in the degree of condensation for wood charcoals at pyrolysis temperatures between 700–1000 °C was also observed using X-ray photoelectron spectroscopy (Nishimiya et al., 1998).

Our data can be related to the conceptual model describing molecular changes in wood derived charcoals produced at 100–700 °C presented in Keiluweit et al. (2010). They determined the chemical structure using near edge X-ray absorption gine structure (NEXAFS) spectroscopy. To describe the molecular changes and increasing degree of condensation in their thermosequence they introduced the terms "transition", "amorphous" and "turbostratic" charcoals. When comparing these conceptual charcoal types to our thermosequence charcoals, the samples produced at 200–300 °C can be described as "transition charcoals". In this part of the thermosequence, we find strongly increasing BPCA contents from 3.3 g BPCA-C/kg OC to 83.2 g BPCA-C/kg OC, indicating an increasing contribution of aromatic C to total OC and the loss of the properties of the original plant biomass. The dominance of B3CA, B4CA and B5CA relative contribution at temperatures up to 600 °C indicates small aromatic units of "amorphous charcoal", while at higher temperatures we find 50% to almost 98% contribution of B6CA, indicating increasing growth of graphene-like structures found in "turbostratic charcoals". Condensation reactions of aromatic rings being the dominating process at these temperatures is also reflected in ongoing decrease of H/C ratios, while O/C ratios remain constantly low at 0.02 (Table 1).

Another approach to obtain information about the degree of condensation of charcoals is the measurement of "ring currents" (Smernik et al., 2006). This method was recently tested on a suite of thermally altered wood and grass at temperatures of 250–850 °C under oxygen limited conditions (McBeath and Smernik, 2009), showing major increase in the degree of condensation between charcoals pyrolyzed at 450 and 850 °C, which fits well the observed increase in B6CA contribution from about 30% at 450 °C to almost 80% at 800 °C in the thermosequence charcoals (Fig. 5).

However, it still remains to be analytically confirmed on identical samples, how these independent methods compare and how they reflect the degree of aromatic condensation.

3.3. Relation to natural charcoal

How do our results compare to real charcoal produced in kilns or during wildfires? Probably our observations on the BPCA yields and patterns could be related to charcoal produced under similar oxygen limited conditions, including charcoal pyrolyzed in traditional kilns or so called biochar, a technical pyrolysis product used in agriculture. Analytical evidence for this statement, however, is still lacking.

It is not clear how our results, obtained on charcoal pyrolyzed under controlled conditions, compare to charcoals produced during wildfires. Typical temperature windows for wildfires as observed in prescribed burning studies would be 275–500 °C at the soil surface (Gimeno-Garcia et al., 2004; Alexis et al., 2007) and up to 800 °C (Alexis et al., 2007) or even higher (Pyne et al., 1996) in the vegetation, meaning the thermosequence we used covered common temperatures relevant for natural fires. The resulting BPCA patterns, however, could be very different depending on the conditions of the wildfire, including wind, gas composition of the atmosphere, duration of the fire event, moisture and source type of biomass. Temperature has been shown to have a strong effect on the degree of condensation and many other physical and chemical properties of charred materials (Shafizadeh and Sekiguchi, 1983; Brown et al., 2006). If indeed temperature dominates aromaticity and degree of condensation of the charcoal formed during wildfires, then the BPCA quantities and patterns observed in our samples could reflect those found in natural charcoals. However, it would be crucial to compare BPCA quantities and patterns of charcoal formed during wildfires.

4. Conclusions

We improved the BPCA method to produce more reliable information about quality and quantity of BC in soils and charcoals and found the following results. Briefly, phthalic acid could replace citric acid as an internal standard, provided contamination with phthalic acid from lab equipment can be excluded. After HNO_3 oxidation soil extracts could be stored under acidic conditions for up to 30 days without affecting quality and quantity of BPCA. After derivatization pure mixtures of BPCA standards mixtures could be stored for months without changes in quantity and can serve as a rapid quality check before GC analysis. As a quality control we used the Chernozem soil reference material from the BC ring trial (Hammes et al., 2007, 2008a).

When we applied the improved method to a charcoal thermosequence (200–1000 °C) of charred wood, BPCA quantities consistently increased up to 700 °C, which are common temperatures for wildfires (Pyne et al., 1996; Gimeno-Garcia et al., 2004; Alexis et al., 2007). Qualitative changes were small over 250–500 °C,

although elemental (C, H, O) composition suggested ongoing condensation up to 1000 °C. Generally, proportions of B6CA reflected the increasing pyrolysis temperature of the charcoal and probably also the increasing degree of aromatic condensation.

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Manuscript 2

Comparison of gas with liquid chromatography for the determination of benzenepolycarboxylic acids as molecular tracers of black carbon

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Comparison of gas with liquid chromatography for the determination of benzenepolycarboxylic acids as molecular tracers of black carbon ☆

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ABSTRACT

Existing methods for black carbon (BC) quantification measure different parts of the BC continuum, which complicates the calculation of a global BC budget. Benzenepolycarboxylic acids (BPCA) are used as molecular markers to quantify and characterize BC in soils and sediments using gas chromatography for BPCA separation (GC-BPCA). Recently, this method was refined for BC analysis in seawater using high performance liquid chromatography (LC-BPCA), which omits the cleaning steps and derivatization necessary in GC analysis. As yet it is not clear whether the two analytical methods yield similar results. Here we apply both methods to a suite of laboratory produced charcoals derived from wood and grass. We found systematically lower total BPCA-C contents and larger analytical variability for all tested charcoals when using GC-BPCA compared to LC-BPCA, the latter giving 1.5 ± 0.3 times higher yields for the charcoal samples formed at 275–700 °C. At lower and higher pyrolysis temperatures the differences between the two analytical methods were larger. The main reason for the differences between the two methods is the loss of BPCA during sample preparation for GC analysis. We propose a correction factor of 1.5 to account for at least part of these losses. No qualitative biases, i.e. towards more or less functionalized BPCAs, were observed between the two methods. The relative contribution of mellitic acid C to total BPCA-C, a measure for the degree of condensation of BC, was the same in the two analytical techniques. Qualitative differences between wood and grass charcoals as detected by both methods were small.

1. Introduction

Black carbon (BC) is known for its persistence in the environment (Goldberg, 1985; Preston and Schmidt, 2006) and can be ubiquitously found in sediments and soils (Schmidt and Noack, 2000; Dickens et al., 2006; Bruun et al., 2008; Nguyen et al., 2009, 2010; Zimmerman, 2010), in water (Hockaday et al., 2006; Koch and Dittmar, 2006) and as aerosol in the atmosphere (Horvath, 1993), thus forming a small but significant part of the global carbon cycle. BC does not have a defined chemical structure but can be regarded as a continuum of thermally altered biomass (Hedges et al., 2000; Schmidt and Noack, 2000; Masiello, 2004). Many methods exist that are designed for detection of different parts of this BC continuum in diverse environmental matrices such as water, sediments and soils, giving disparate results for identical samples (Hammes et al., 2007). As a consequence, BC data obtained by these different methods for different environmental compartments are not easy to compare, which hampers the calculation of a global BC budget (Schmidt and Noack, 2000; Forbes et al., 2006).

The molecular marker method of benzenepolycarboxylic acids (BPCA) analysis (Glaser et al., 1998; Brodowski et al., 2005; Schneider et al., 2010) does provide both a quantitative and a qualitative measure of BC, and has been widely applied to characterize BC in soils (Glaser et al., 2000; Czimczik et al., 2003; Glaser and Amelung, 2003; Rodionov et al., 2006, 2010; Brodowski et al., 2007; Guggenberger et al., 2008; Hammes et al., 2008b) and charcoals (Kaal et al., 2008; Schneider et al., 2010). Soils are digested with nitric acid during which the BC present in the sample is converted to a suite of BPCAs. Before analysis of the BPCAs by gas chromatography-flame ionization detection (GC-FID) the extract needs to be cleaned in several steps and derivatized.

An alternative BPCA method was recently introduced for seawater samples that uses high performance liquid chromatography

☆ The study was proposed by MPWS, TD and MWIS. TD provided his technical expertise for BPCA measurements on HPLC. RHS and MPWS carried out the experiments and data analysis. Paper writing was completed by MPWS under the supervision of MWIS, with contributions of all co-authors.

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coupled to diode array detection (HPLC-DAD) (Dittmar, 2008; Dittmar and Paeng, 2009). This method omits the cleaning steps and derivatization necessary for GC-FID measurements.

However, a test of how results of the modified method compare with results obtained by the traditional method is still lacking. In this paper we test and compare both methods to identify and quantify BPCA. We chose two laboratory produced charcoal thermosequences made from wood and grass, because charcoal is an important source of BC in the environment (Schmidt and Noack, 2000). By choosing temperatures from 200 to 1000 °C and two sources of biomass we cover a broad range of different properties, such as elemental composition and degree of condensation (Schneider et al., 2010). Furthermore, we discuss the qualitative information that the BPCA method provides and compare the BPCA data with respect to the two types of biomass.

2. Materials and methods

2.1. Charcoal thermosequences

Charcoals were produced from chestnut (*Castanea sativa*) wood chips (from here: wood) and rice straw (*Oryza sativa*) pieces (from here: grass), at temperatures from 200–1000 °C as described in Schneider et al. (2010). Both materials originate from Ticino, Switzerland (Hammes et al., 2006). The charcoal thermosequences include the reference wood and grass charcoal pyrolyzed at 450 °C that was used in a broad BC comparison study, where several laboratories were involved and a wide range of methods covered (Hammes et al., 2007). Elemental analysis (CHO) of the charcoals was carried out on a LECO CHN-900 and a LECO RO-478 (Mönchengladbach, Germany) in two laboratory replicates.

2.2. Sample preparation and chromatographic analysis

A summary of both methods, BPCA analysis by GC-FID and by HPLC-DAD, can be found in Table 1. All samples were prepared in three replicates.

Charcoal samples were prepared for BPCA analysis by GC-FID (from here: GC-BPCA) as outlined by Schneider et al. (2010), based on the method described in Glaser et al. (1998) and Brodowski et al. (2005). All BPCA peaks were baseline separated (Fig. 1a). Peak identification was based on mass spectra and retention times (Glaser et al., 1998; Brodowski et al., 2005).

For the analysis of BPCA by HPLC-DAD (from here: LC-BPCA) the various cleaning steps that are needed before GC-FID analysis could be omitted (Table 1). This significantly simplified the work-up procedure (Dittmar, 2008), while sample amounts could also be reduced by up to a factor of 10 compared to GC-BPCA (Table 1).

For LC-BPCA, 2–5 mg C of charcoal samples were weighed into quartz digestion tubes, 2 ml of 65% HNO_3 was added and samples were digested at 170 °C for 8 h in a pressure digestion apparatus (Schramel et al., 1980). After digestion, aliquots of 1 ml were dried under a stream of nitrogen gas and dissolved in 1 ml of a mixture of methanol (MeOH) and water (25:75 v:v) and further diluted by transferring aliquots (20–100 µl) to HPLC vials that were then filled to 1 ml with mobile phase A (Table 1). BPCA separation was carried out on a Thermo Surveyor HPLC instrument equipped with a Waters Atlantis T3 column (150 mm × 2.1 mm, 3 µm) applying a gradient of phosphate buffer (pH 8) modified with tetrabutylammonium bromide (TBAB) (2 g/l) and MeOH over 48 min (Tables 1 and 2). A photo diode array detector (DAD) was used for peak identification (absorbance spectra 220–380 nm) and quantification

Table 1
Specifications for sample preparation and for chromatographic analysis of benzene polycarboxylic acids (BPCA), using either gas chromatography with flame ionization detector (GC-BPCA) or high performance liquid chromatography with diode array detector (LC-BPCA).

Nr	Work step/description	GC-BPCA	LC-BPCA
1	Sample preparation before HNO_3 digestion	Trifluoroacetic acid (TFA) digestion (4 h at 105 °C), filtration: collect sample on glass fiber filter (GF 6, Schleicher and Schuell, Dassel, Germany) and rinse with excess of water, dry (2 h at 40 °C)	–
2	HNO_3 digestion, conversion to BPCA Solid to acid ratio, mg charcoal-C ml^{-1} HNO_3	2 ml 65% HNO_3 (8 h at 170 °C in oven) 1–25	2 ml 65% HNO_3 (8 h at 170 °C in oven) 1–2.5
3	Sample preparation after HNO_3 digestion	Filtration over ashless cellulose filter (589/3, 110 mm diameter, Schleicher and Schuell, Dassel, Germany) into 10 ml volumetric flasks, fill up with deionized water	Drying at 60 °C under N_2 stream and dissolution in methanol/water (1:3), further dilution with mobile phase A
4		Addition of internal standard phthalic acid, cleaning with cation exchange resin (Dowex 50 W X 8, 200–400 mesh, Fluka, Steinheim, Germany), freeze drying for acid removal, transfer to GC vials with four times 1 ml methanol	
5	Derivatization	100 µl BSTFA + TMCS, 100 µl pyridine (2 h at 80 °C + storage for 24 h)	–
6	Chromatographic analysis		
	Mobile phase A	He	Ortho phosphoric acid (50%) 1 ml l^{-1} Tetrabutylammonium bromide (TBAB) 2 g l^{-1} – Dissolved in water – Adjusted to pH 8 by slowly adding 1 M NaOH
	Mobile phase B	–	Mobile phase A + 75% MeOH
	Injection volume	1 µl	20 µl
	Injections per sample replicate	2	1
	Flow rate	0.8 ml min^{-1}	0.18 ml min^{-1}
	Column temperature	100–300 °C	16 °C
	Column/quantification	Agilent DB-5 (50 m, diameter 0.2 mm)/flame ionization detector (FID)	Waters Atlantis T3 3 µm (150 mm, diameter 2.1 mm)/ UV absorption at 240 nm
7	Identification	Retention time, GC-MS	Retention time, absorbance spectra 220–380 nm
	Standard error for BPCA-C kg^{-1} OC (%)	Mean: 6.5; min: 1.3; max: 34.8	Mean: 1.6; min: 0.3; max: 4.5

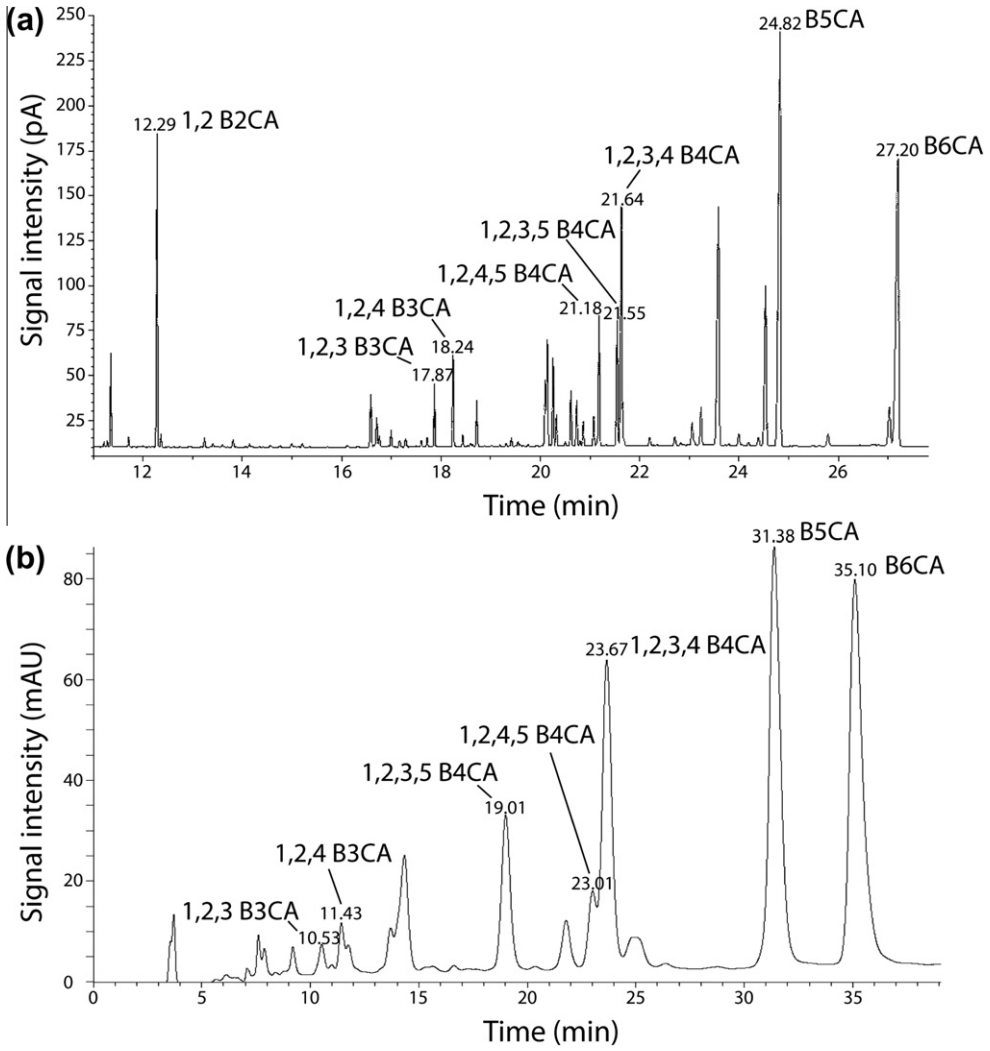

Fig. 1. Chromatogram of nitric acid oxidation products of grass pyrolyzed at 450 °C obtained by (a) GC-FID and (b) HPLC-DAD. Peak identification of black carbon molecular markers (1,2,3-; 1,2,4-B3CA, 1,2,3,5-; 1,2,4,5-; 1,2,3,4-B4CA; B5CA; B6CA) for HPLC-DAD is based on absorbance spectra (220–380 nm) and retention time, for GC-FID it is based on mass spectra and retention time. Phthalic acid (1,2-B2CA) is used as an internal standard for GC-FID analyses.

Table 2
Mobile phase mixing gradients of the LC-BPCA analytical method. For mobile phase composition see Table 1.

Time (min)	Mobile phase B (vol%)
0.01	Start
0.02	40
48	47
49	100
53	100
54	40
64	40
64.01	Stop

(absorption at 240 nm) operated with XCalibur software (v. 2.3.x). Standard solutions of BPCA were used to generate a calibration curve and as a reference for UV absorption spectra. Identification was also based on the absorbance spectra as described in Dittmar (2008).

For this study, the mixing gradient between mobile phase A and B was optimized for fastest separation (Table 2). This compromised some of the separation, as not all of the benzene tricarboxylic acid (B3CA) and benzene tetracarboxylic acid (B4CA) isomers were baseline separated (Fig. 1b). However, the contribution of hemimellitic (1,2,3-B3CA) and trimellitic (1,2,4-B3CA) acid to total BPCA-C is low in most samples (see B3CA in Figs. 2 and 3), and thus the faster procedure did not introduce a large error. The peaks of prehnitic acid (1,2,3,4-B4CA) and pyromellitic acid (1,2,4,5-B4CA) were quantified together with mellophanic acid (1,2,3,5-B4CA) as B4CA. Compounds that contributed most to total BPCA, benzene pentacarboxylic acid (B5CA) and mellitic acid (B6CA) were baseline separated with the faster separation gradient (Fig. 1b). If necessary, the mixing gradient of the mobile phases can be optimized for baseline separation of all peaks, as shown by Dittmar (2008).

2.3. Statistics

All statistical analyses were performed with SPSS software package version 17.0. For the comparison of means we applied a one-way ANOVA ($p < 0.05$).

3. Results and discussion

3.1. Elemental characterization of charcoals

Fig. 4 shows the H/C and O/C ratios for the charcoals derived from grass and wood at pyrolysis temperatures ranging from 200–1000 °C. With increasing pyrolysis temperature the H/C and

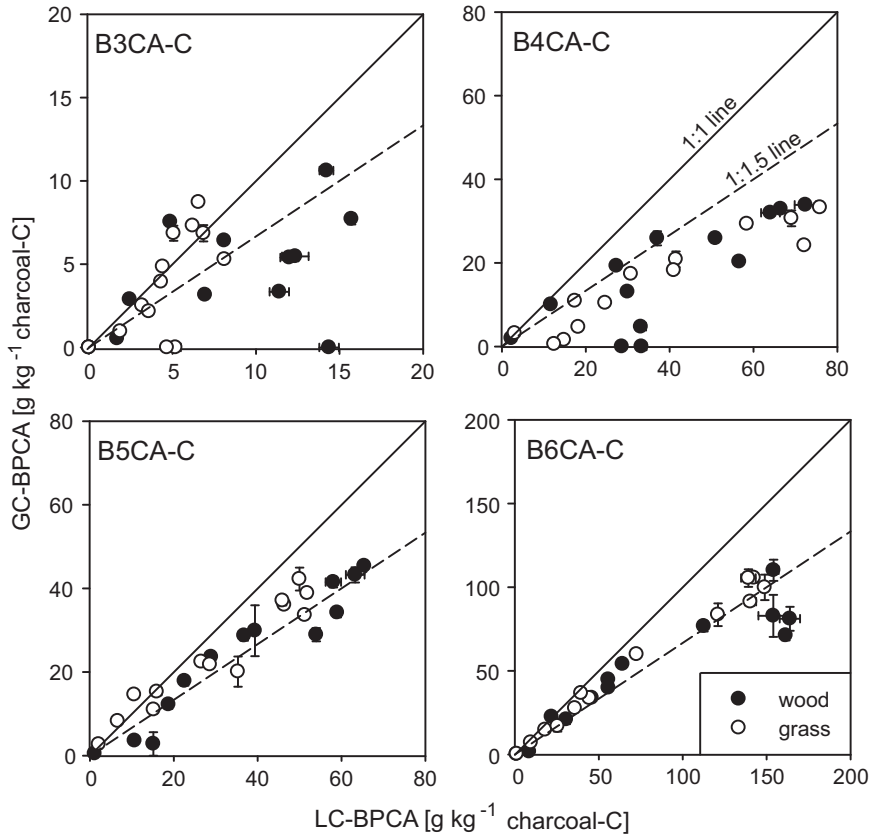

Fig. 2. Comparison of marker molecule contents (B3CA-, B4CA-, B5CA-, B6CA-C per charcoal-C) in thermosequence charcoals as obtained by two different methods, using either gas chromatography with flame ionization detector (GC-BPCA, *y*-axis) or high performance liquid chromatography with diode array detection (LC-BPCA, *x*-axis). The thermosequence comprises chars derived from two sources of biomass (wood and grass) pyrolyzed at 13 temperatures from 200 to 1000 °C. For easier comparison the 1:1 (solid) and 1:1.5 line (dotted) are shown. Please note the different scaling of the plots.

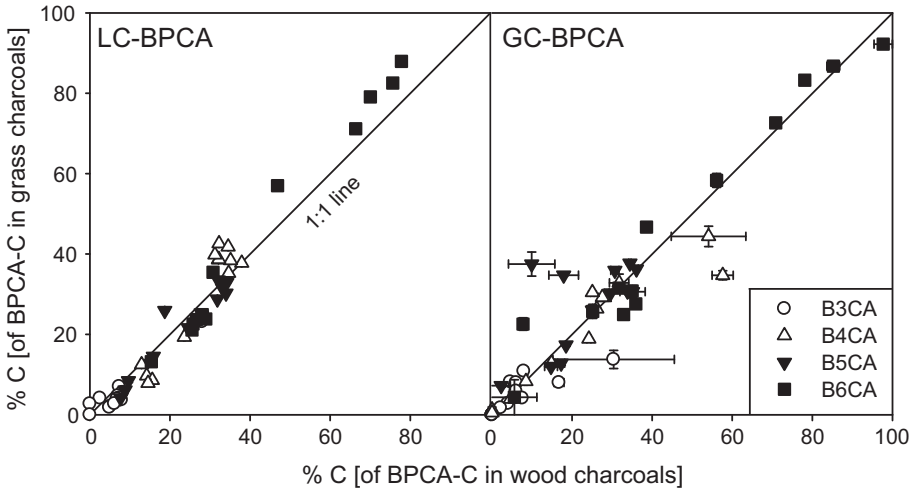

Fig. 3. Quality of charcoals as indicated by the relative contribution of individual molecular markers for black carbon (B3CA, B4CA, B5CA, B6CA) to total BPCA-C in wood (*x*-axis) and grass (*y*-axis) pyrolyzed at temperatures from 200 °C to 1000 °C, as detected with high performance liquid chromatography – diode array detector (LC-BPCA, left) or with gas chromatography – flame ionization detector (GC-BPCA, right).

O/C ratios of the charcoals became smaller, irrespective of the type of precursor biomass. The observed trends indicate typical reactions during pyrolysis, like initial dehydration and demethylation, and decarboxylation at higher temperatures, as described previously (Baldock and Smernik, 2002; Almendros et al., 2003; Antal and Gronli, 2003; Hammes et al., 2006; Keiluweit et al., 2010; Zimmerman, 2010).

At temperatures of 500 °C and higher, differences in the H/C ratios were very small between charcoals derived from different biomass sources at the same pyrolysis temperature. In the low temperature range (200–275 °C), grass charcoals had a lower O/C ratio than wood charcoals, while in the high temperature range (600–1000 °C), wood charcoal O/C ratios were relatively low (close to 0). The higher O/C ratios for grass charcoals at these

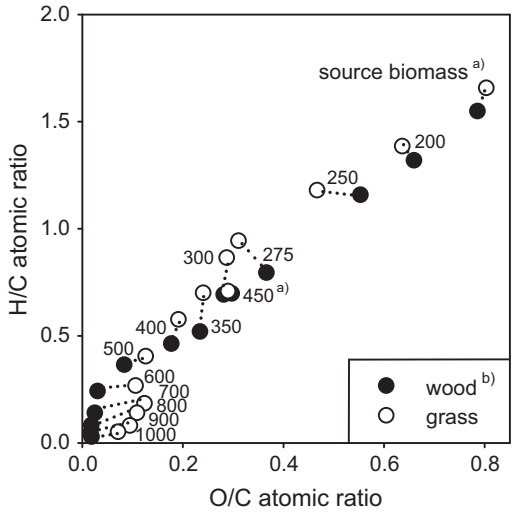

Fig. 4. Van Krevelen plot of pyrolyzed chestnut wood (*Castanea sativa*) and rice grass (*Oryza sativa*), including the source biomasses. Grass and wood samples pyrolized at the same temperature are connected with a dotted line, numbers indicate the respective pyrolysis temperatures (200–1000 °C). (a) Data taken from Hammes et al. (2006). (b) Data taken from Schneider et al. (2010).

temperatures may be related to the presence of phytoliths, typically found in *O. sativa* (Zheng et al., 2003; Itzstein-Davey et al., 2007) and to a generally greater degree of aromatic condensation in wood charcoal compared to grass charcoal (Hammes et al., 2008a; Keiluweit et al., 2010).

The H/C and O/C ratios of the 450 °C reference charcoals (Hammes et al., 2006) are higher compared to what could be expected given the values for the 400 °C and 500 °C charcoals (Fig. 4). Hammes et al. (2006) used wood sticks (40 × 5 × 5 cm) and bundled rice straw for pyrolysis, instead of wood chips and cut rice straw pieces used in this study. The larger pieces probably resulted in some self insulation of the sticks and bundles and consequently in lower average pyrolysis temperatures and a smaller degree of charring in the core of the sticks and bundles. This assumption is in line with nuclear magnetic resonance observations in Hammes et al. (2008a) showing the presence of cellulose after heating.

3.2. Comparison of methods

3.2.1. Total yields of BPCA in thermosequence charcoals

The results for total BPCA-C content per kg charcoal-C, are shown in Fig. 5a and b, either quantified by GC-BPCA (a) or LC-BPCA (b). Irrespective of the method total BPCA-C production increased strongly with pyrolysis temperature up to 400 °C and then leveled off (Fig. 5). At a temperature of 600 °C a maximum of 242 and 212 g/kg was reached for wood and grass charcoal, respectively (Fig. 5b). Compared to the results from LC-BPCA, the BPCA-C contents obtained by GC-BPCA follow the same pattern (Fig. 5a), but on a lower level.

In general, differences between the two methods were largest for low temperature (200 °C and 250 °C) and high temperature (>700 °C) charcoals, and smallest for charcoal produced at intermediate temperatures. Over the whole range, we found 1.1–2.6 times more BPCA-C by LC-BPCA compared to GC-BPCA. This factor was relatively constant at 1.2–1.8 (mean of 1.5) over the broad intermediate temperature range of 275–700 °C, where probably most natural and artificial charcoals are produced (Pyne et al., 1996; Gimeno-Garcia et al., 2004; Alexis et al., 2007).

The additional steps in the preparation process (Table 1) are most likely the cause for the overall lower BPCA-C contents obtained by GC-BPCA. The trifluoroacetic acid (TFA) pretreatment (step 1, Table 1) and the cleaning with cation exchange resin (step 4, Table 1) were evaluated by Ziolkowski and Druffel (2009). For several materials containing condensed aromatic C forms (fullerenes, carbon lamp black, soot, carbon nano tubes) they found that TFA and cation exchange resin treatment of the samples resulted in up to 10% lower C recoveries when compared to C recoveries obtained for samples without these steps. They explain the losses due to the additional sample handling. By comparison, sample handling can thus explain part of the lower GC-BPCA yields compared to LC-BPCA.

In order to identify other potential causes, we conducted several tests to identify which other steps may contribute the most to the observed losses.

First, we evaluated differences in the nitric acid digestion (step 2 in Table 1). For some samples more material was used in the digestion step for GC-BPCA compared to LC-BPCA samples (Table 1), and it is possible that higher solid to acid ratios, i.e. acid limitation, could lead to incomplete conversion of BC to BPCA. We compared two different amounts of charcoal-OC at constant nitric acid

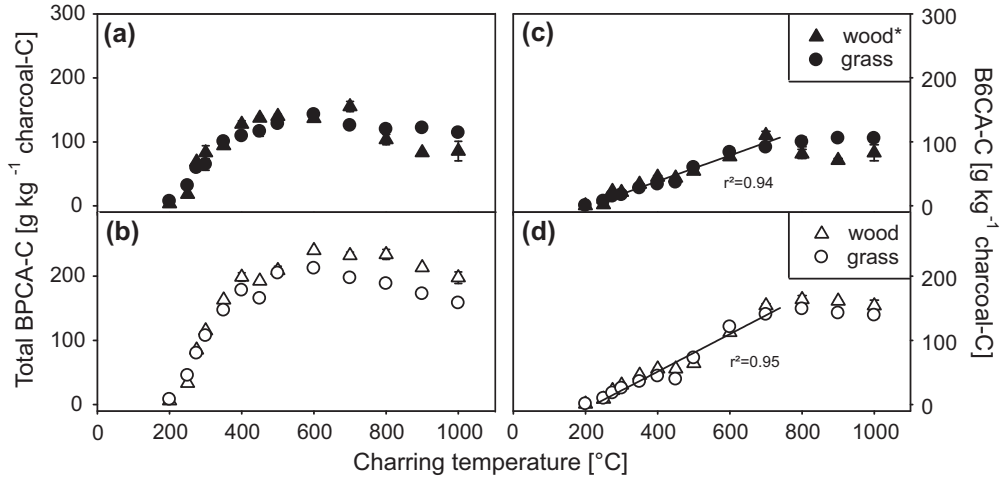

Fig. 5. BPCA-C content per charcoal-C in wood (triangles) and grass (circles) pyrolyzed between 200 and 1000 °C, using either (a) gas chromatography with flame ionization detector (GC-BPCA) or (b) high performance liquid chromatography with diode array detector (LC-BPCA) for separation and quantification of BPCA; B6CA-C content per charcoal-C for the same samples obtained by two alternative methods for quantification of B6CA, (c) GC-BPCA or (d), LC-BPCA. The linear regressions are based on data from 250 °C to 700 °C. Error bars indicate standard errors ($n = 3$). *Data taken from Schneider et al. (2010).

volume (2 ml HNO_3 65% m/m) but did not detect differences in the recovery of BPCA-C (measured using GC-BPCA) from charcoal: 11 mg charcoal-C gave a total yield of 1.02 mg BPCA-C (SE = 0.07), i.e. a BPCA-C content of 94 g/kg C (SE = 4.7); 2 mg charcoal-C gave a total yield of 0.17 mg BPCA-C (SE = 0.03), i.e. 86 g/kg C (SE = 15.3). Thus, the observed differences between GC-BPCA and LC-BPCA can probably not be explained by acid limitation or other differences in the digestion step.

Second, we evaluated the filtration step after nitric acid oxidation (step 3 in Table 1). Losses in this step would not be monitored by an internal standard, because internal standard is added only later in the preparation process. When tested with a standard mixture (1,2,3-B3CA, 1,2,4-B3CA, 1,2,3,5-B4CA, B5CA, B6CA) 22% losses (SE = 3.6) were detected, which could explain a large part of the observed differences between the two methods. A possible improvement in the protocol for BPCA analysis is to use 50 ml for rinsing the filter instead of 10 ml (step 3 in Table 1).

Third, the derivatization (step 5), needed for GC-BPCA but not for LC-BPCA, can be incomplete for BPCA molecular markers due to matrix effects. To test this, BPCA standards were added to a wood 450 °C nitric acid extract before derivatization, at different concentrations. Based on the data obtained by standard addition a linear regression was calculated and quantified amounts were compared to the amounts obtained by external standard quantification. The differences for the tested BPCA were small and not significant (data not shown). Thus, matrix effects on derivatization cannot explain the observed differences between the two methods.

At last, we compared the quality of the external standard regressions that are used for quantification of BPCA. The r^2 value of the regressions applied to the external standard series ranged from 0.998 to 0.999 for LC-BPCA quantification. For GC-BPCA the corresponding r^2 value varied between 0.982 and 0.998, and this method thus has a slightly higher uncertainty for BPCA quantification, as can also be seen from higher standard errors (Table 1, Fig. 3). However, the quality of the regression cannot explain the systematically lower concentrations found with GC-BPCA.

In summary, the lower BPCA-C contents observed with the GC-BPCA method, compared to LC-BPCA, appear to be primarily caused by losses during sample preparation, namely in steps 1, 3 and 4 (Table 1). A possible way to monitor losses over the whole procedure could be to run anthracene (or another PAH) parallel to unknown samples and use it as an external standard to account for the losses, similar to the proposal of Ziolkowski (2009). However, it would still require additional testing to determine if PAH behave same as the condensed aromatic structures present in diverse samples such as sediments, soils and charcoals. Advantages and disadvantages of the two methods are summarized in Table 3.

Table 3
Advantages and disadvantages of the two methods for BPCA analysis.

GC-BPCA	LC-BPCA
+ Proven for BC analysis in soil and sediment samples with mineral matrix	+ Tested for BC analysis in charcoals and water samples – Samples with mineral matrices not yet tested
– Derivatization necessary	+ No derivatization
– Time consuming cleaning procedure	+ Quick sample preparation
+ Shorter run time than HPLC,	– Longer run time than GC, per analysis
– Two injections per sample necessary, resulting in lower throughput	+ Higher sample throughput
– Higher losses during sample processing	+ Higher reproducibility

3.2.2. Detection of individual BPCA in thermosequence charcoals

A comparison of individual BPCA marker molecules (B3CA, B4CA, B5CA, B6CA) detected by either GC-BPCA or LC-BPCA is shown in Fig. 2. Data points close to the 1:1 line indicate that both methods resulted in similar amounts of a molecular marker in the same sample. GC-BPCA resulted sometimes in relatively higher B3CA contents compared to LC-BPCA, but B3CAs are less abundant. For the quantitatively important B5CA and B6CA most data points fall in between the 1:1 and the 1:1.5 line, while most of B4CA data points show >1.5 times higher contents when analyzed by LC-BPCA. In general, however, we did not observe major differences between the individual molecular markers. Thus, the relative contribution of individual marker molecules to total BPCA, which is an indicator for the degree of thermal alteration (Glaser et al., 1998; Schneider et al., 2010), are comparable between the two methods.

3.2.3. Influence of biomass on total BPCA yields and relative distributions

Comparing the two sources of biomass, wood charcoals always yielded slightly higher BPCA-C contents than grass charcoals, except for the lowest pyrolysis temperatures 200 and 250 °C (Fig. 5b). Here the BPCA-C content was about one third higher in grass compared to wood charcoals. At temperatures of 275–1000 °C, grass charcoals showed on average 11.5% lower BPCA-C contents compared to wood charcoals. The reasons for the observed differences can be explained by the higher lignin content in wood compared to grass, which is a precursor molecule for the formation of condensed aromatic structures (Mok et al., 1992; Czimczik et al., 2002).

To test if the precursor biomass affects the yields of individual BPCA, we compared yields obtained by both methods in a cross plot (Fig. 3). Differences were small in terms of BPCA quality when individual BPCA molecular markers are normalized to total BPCA-C for both methods (Fig. 3). Data points for all individual molecular markers obtained by LC-BPCA group around the 1:1 line, thus showing that the type of precursor biomass does not influence the BPCA distributions. The same was observed for the GC-BPCA data. However, some outliers, with high standard errors, indicate that greater uncertainties are associated with the GC-BPCA method. To conclude, pyrolysis temperature appears to be the controlling factor that determines BPCA distributions, irrespective of biomass source or analytical method.

3.2.4. B6CA content as indicator of condensation reactions

The B6CA-C content per kg charcoal-C is shown in Fig. 5c and d, quantified by either GC-BPCA (c) or LC-BPCA (d). With both methods we found a linear increase in the B6CA-C contribution in the intermediate temperature range (250–700 °C) reflecting the formation of more condensed aromatic structures with increasing pyrolysis temperature. The B6CA-C content per kg charcoal C could thus serve as a good single value estimate for the degree of condensation for both sources of biomass, grass and wood, and possibly also for other types of charcoal produced under similar conditions.

An alternative measure of the degree of condensation is the average number of carboxylic groups, calculated by quantifying the number of carboxylic groups substituted on the benzene rings (3–6 acids) weighted by their relative contribution to total BPCA (Ziolkowski and Druffel, 2010, auxiliary material S1). Applied to the reference charcoals (450 °C) we found 4.9 and 4.7 for wood and grass, respectively (Table 4), which is in agreement with Ziolkowski and Druffel (2010) for the reference charcoals (4.8 and 4.7). The average number of acids in the thermosequence range from 4.2 (wood) and 4.3 (grass) at 200 °C to 5.6 (wood) and 5.8 (grass) at 1000 °C (Table 4). However, the average number of acids remains constant at 4.7–4.8 over a broad temperature range (250–450 °C). This means B6CA-C as % of charcoal-C is more

Table 4
Average number of acid groups in BPCA for charcoals produced at different temperatures (in °C). For all samples standard error is <0.02 (n = 3).

	200	250	275	300	350	400	450	500	600	700	800	900	1000
Wood	4.2	4.8	4.8	4.8	4.8	4.8	4.9	4.8	5.1	5.4	5.4	5.6	5.6
Grass	4.3	4.7	4.7	4.8	4.8	4.7	4.7	4.9	5.3	5.6	5.6	5.7	5.8

sensitive as a condensation indicator than the average number of acids, and also more sensitive than B6CA-C as % of total BPCA-C (Schneider et al., 2010), to reflect the molecular changes taking place in this temperature range, which are indicated by elemental data (Fig. 4) and by increasing BPCA-C contents (Fig. 5). We have to note, however, that if the amount of charcoal-C is not known a priori before BPCA analysis, e.g. in BC containing soils, one cannot readily express B6CA contents as % of charcoal-C.

With both GC and LC methods we found total BPCA-C as well as B6CA-C contents to slightly decrease at temperatures higher than 600 °C (Fig. 5a and b). Theory and experimental observations indicate that the observed decrease likely lies in the structure and degree of condensation of the BC molecules: An 'infinite' fully condensed structure (i.e. graphite) would produce solely B6CA. Then, based on theoretical calculation, the maximum recovery of C would be 67%, while a polyacene structure with all rings sharing two C atoms with both neighbors would produce only B4CA with an optimal recovery of 83%. That means for more condensed structures, producing mainly B6CA, less of the aromatic C is converted into BPCA-C, resulting in a decrease in the total BPCA-C content. However, the recovery is also dependent on the distribution of the various BPCAs, something that is not fully predictable, and may result in lower recoveries (Dittmar, 2008).

Similar to elemental data, we found the less intense pyrolysis conditions of the 450 °C charcoals, produced for the BC ring trial, reflected in lower B6CA-C contents compared to expected values when the contents of adjacent samples are considered. This effect was also detectable for total BPCA-C contents in the 450 °C charcoals by LC-BPCA (Fig. 5b).

4. Conclusion

Comparing the two methods for BPCA detection, it appears that there are more sources of errors and higher systematic losses using the GC-BPCA method because of the various cleaning and other procedural steps involved. In contrast, the LC-BPCA method proved to be a more robust quantification technique for BPCA, mainly because of the minimum degree of sample preparation. However, it has so far only been tested for completely organic samples, and samples with a mineral matrix such as soil and sediment samples, may introduce analytical problems. Additional testing needs to be performed to come to a common LC-based method for the characterization of BC in the biggest pools of BC: oceans, sediments and soils (Forbes et al., 2006; Preston and Schmidt, 2006; Dittmar and Paeng, 2009).

The LC-BPCA method detected 1.5 ± 0.3 times more BPCA-C compared to the GC-BPCA method for charcoals produced at 275–700 °C. In order to compare previous GC-with LC-BPCA data we propose a correction factor of 1.5 to account for losses during sample preparation before GC analysis. If our observation holds true for soil and sediment samples, the implications for the calculation of the BC cycle may be profound. The systematic offset observed between the two methods must be taken into account when comparing existing BPCA-C data, e.g. in oceans (measured by LC-BPCA) and soils (measured by GC-BPCA).

It is important to note that a factor of 1.5 would equalize GC- and LC-BPCA data, but is not intended to replace the conversion factor of 2.27 (Glaser et al., 1998), which had been introduced to correct for method-inherent carbon losses during nitric acid oxidation. Also, we dissuade from using the conversion factor 2.27 at all, because it was not reproducible in other studies and its general usefulness had been questioned (Brodowski et al., 2005; Ziolkowski and Druffel, 2009; Schneider et al., 2010). For future studies we recommend to report BPCA-C data always "as measured" in order to facilitate comparability of data between studies and then apply the correction factor of 1.5 where necessary.

Future studies should also carefully evaluate the filtration step after nitric acid oxidation, because that is probably the largest source of error during GC analysis. Possible improvements in the protocol for the GC-BPCA method include increasing the amount of water to rinse the filter (step 3, Table 1) and using anthracene as an external standard to account for losses over the whole sample preparation procedure. However, whether these measures are suitable to improve the GC-BPCA method has not yet been tested.

The B6CA-C yield was highly correlated to charring temperature up to 700 °C, both in grass and wood charcoal. Consequently, B6CA-C yields may not only provide information about the degree of condensation in charcoal but can potentially also be used to infer the formation temperature in thermally altered biomass with long exposure times, like charcoals produced in traditional kilns or so called biochars.

We observed only minor differences in quantity and quality of BPCA produced from two different biomass sources, grass and wood. In contrast, pyrolysis temperature determined BPCA patterns. Both analytical methods were capable to detect these systematic patterns in our samples, indicating that both methods are a reliable tool for black carbon studies.

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Manuscript 3

Pyrogenic carbon soluble fraction is larger and more aromatic in aged than in fresh charcoal

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Short Communication

Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal

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ABSTRACT

Recent studies show that pyrogenic matter is one of the most stable compounds in the soil but less inert than previously expected. One potential pathway yielding losses from soil is solubilisation of pyrogenic compounds. In batch experiments, we estimated the proportion and molecular composition of soluble (<0.45 μm) and colloidal fractions (0.45–5 μm) extractable from a freshly pyrolysed charcoal and a 10 year old wildfire charcoal. These fractions represented a very small fraction (<2.7 g kg^{-1}) of chars. The benzene polycarboxylic acids (BPCA) pattern indicated that 40–55 times more condensed structures were released from the aged char than from the fresh char. This study shows that the soluble fraction of the char is small, and tends to increase with the residence time in the soil.

1. Introduction

Fire-derived organic compounds, also known as pyrogenic carbon (PyC), are ubiquitous in soils and sediments (Schmidt and Noack, 2000) and riverine and oceanic dissolved organic matter (Kim et al., 2004; Dittmar, 2008; Dittmar and Paeng, 2009). Due to its chemical recalcitrance, PyC has been considered as particularly stable in soil (Skjemstad et al., 1996). However, recent studies have suggested that PyC might disappear from soil profiles faster than previously expected (Hammes et al., 2008; Kuzyakov et al., 2009).

Potential mechanisms for PyC losses from soil could be either biotic or abiotic oxidation (Zimmerman, 2010; Cheng et al., 2006), and/or transport by lixiviation or erosion (Rumpel et al., 2006).

In fact, substantial amounts of PyC have been found in riverine and oceanic dissolved organic matter, using either specific molecular markers techniques (Dittmar, 2008) or ultrahigh resolution mass spectrometry (Kim et al., 2004; Hockaday et al., 2006). Mechanisms of transport from soil to river, however, are yet unknown.

According to estimations based on literature, over 80% of the PyC produced gets incorporated into soil (Preston and Schmidt, 2006). PyC probably becomes soluble as the surface becomes increasingly more oxidised (Lehmann et al., 2005). Circumstantial evidence suggests that PyC can be transported by water through the soil profile. Bird et al. (1999) observed that small PyC particles could be transported into underlying soil horizons. Guggenberger et al. (2008) also found an accumulation of PyC-specific molecular biomarkers in lower layers of permafrost. It is, however, not clear how much of the bulk pyrogenic matter becomes soluble during degradation.

The objectives of this study are to estimate the amounts of the potentially soluble and particulate fractions that can be released from a fresh charcoal and from an oxidised 10 year old charcoal, and identify the molecular marker patterns of the leachate.

2. Materials and methods

The fresh char was pyrolysed at 450 °C for 5 h under N_2 from chestnut wood (*Castanea sativa*) according to Hammes et al. (2006). The aged char was collected from an experimental forest fire where chestnut trees had been burned 10 years ago (Prometheus site, Ticino, Switzerland) (Wüthrich et al., 2002). Charcoal pieces (>5 cm) were collected on the soil surface of the plots and gently cleaned using soft brush (in dry state) to remove soil particles attached to it. These charcoal pieces had been in contact with air and soil and therefore we assumed them to be more oxidised than the fresh char. Both chars were produced from the same feedstock and to comparable temperature (wildfire char temperature of 450 °C according to Turney et al., 2006). To homogenize the samples, both fresh and aged chars were air-dried, ground and sieved through a 1 mm sieve.

In batch experiments similar to Kaiser et al. (2001), we tested different ratios of char mass to water volume and shaking

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durations. The combination which produced the largest concentrations of soluble mass was obtained at the ratio of 8 g bulk dry material to 100 ml deionised water, shaken for 6 h. Each treatment was repeated 6 times.

The soluble and colloidal fractions were collected by vacuum filtration (0.45 and 0.45–5 μm), freeze-dried, weighed and analysed for carbon (C) and nitrogen (N) (Vario EL, Elementar Analysis systems, Hanau, Germany).

The benzene polycarboxylic acids (BPCA) molecular marker method was employed to quantify and characterize the PyC in the soluble and colloidal fraction (Glaser et al., 1998; Brodowski et al., 2005; Schneider et al., 2010). Briefly, samples ($n = 3$) were pretreated with 4 M trifluroacetic acid (4 h, 105 °C), followed by conversion of PyC into BPCA by nitric acid oxidation (8 h, 170 °C). The digested extract was purified using cation exchange resin, freeze-dried and subsequently derivatised and analysed on a gas chromatograph equipped with a flame ionization detector. The acids with 3, 4, 5 and 6 carboxyl functions (B3CA, B4CA, B5CA and B6CA, respectively) were identified, quantified and summed up to represent the amount of pyrogenic molecular markers in the material.

3. Results and discussion

The total mass of soluble and colloidal fractions extractable in optimised batch experiments from both fresh and aged chars was small (<0.3% mass of the initial; Table 1). Under field conditions, these chars probably would release even less PyC. Major et al. (2010) also observed that two years after the input of charcoal to the soil, soluble PyC fluxes represented less than 1% of the annual PyC budget.

The elemental composition shows that the soluble and colloidal fractions were made up of relatively less carbon (<50% C) and more nitrogen (>0.6% N), than the bulk material (>67% C and <0.36% N). The nitrogen concentrations were particularly large for both colloidal and soluble fractions of the aged char (>1.80% N). C to N ratios of both char colloidal and soluble fractions (50 and 55 for fresh char and 15 and 18 for the aged char) were very small. This could either correspond to components which have not been pyrolysed or rich-N pyrolysed compounds which are preferentially soluble. The large N content in the aged char could be also due to microbial biomass that is present on the surface of old charcoal pieces (Hockaday et al., 2007) or sorbed dissolved organic matter accumulated along time.

The BPCA markers produced by both chars (125.7 ± 8.1 and 171.6 ± 6.0 g BPCA-C kg^{-1} OC for fresh and aged char) were in line with previous findings (Hammes et al., 2007). Also, the relative proportions of BPCA markers were similar, with slightly more B3CA in the aged char than in the fresh char.

Although the total mass of soluble and colloidal fractions was similar for both chars, the BPCA markers were 40–55 times more abundant in the soluble and colloidal fractions of aged char. We attribute this difference to the biotic or/and abiotic oxidation of the aged char which could lead to the production of soluble PyC clusters.

Table 1
Mass, C and N content and BPCA content in the soluble and colloidal fractions and in the bulk fresh and aged chars, respectively. Mean values in a column within each type of char followed by the different letters are significantly different at $P < 0.05$ using post-hoc analysis Least Significant Difference.

	Mass g kg^{-1}	C g kg^{-1}	N g kg^{-1}	C:N	ΣBPCA gC kg^{-1} OC
Fresh char					
Bulk	1000 ± 0.0a	667 ± 40a	1.0 ± 0.1b	695a	125.7 ± 8.1a
Soluble	1.5 ± 0.1c	374 ± 19b	7.5 ± 0.7a	50b	1.0 ± 0.1b
Colloidal	2.7 ± 0.2b	365 ± 11b	6.7 ± 0.5a	55b	1.3 ± 0.6b
Aged char					
Bulk	1000 ± 0.0a	669 ± 6a	3.6 ± 0.1b	184a	171.6 ± 6.0a
Soluble	1.4 ± 0.1b	294 ± 9b	19.9 ± 1.8a	15b	41.0 ± 0.7c
Colloidal	1.7 ± 0.1b	319 ± 8b	18.0 ± 0.1a	18b	74.0 ± 1.9b

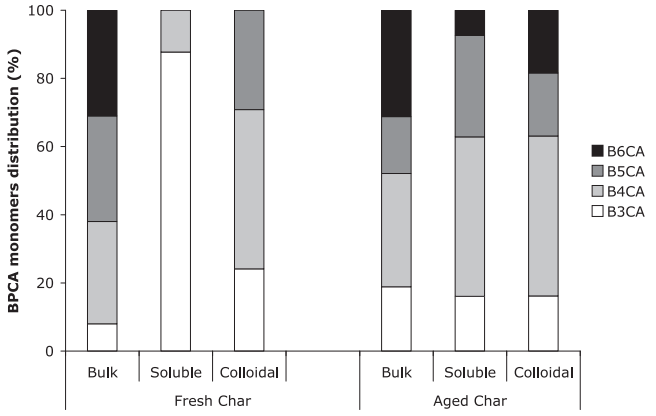

Fig. 1. BPCA monomers distribution (%) of fresh and aged chars, shown for bulk char and soluble and colloidal fractions.

The relative contributions of individual molecular markers reflect the quality of PyC and the size of the aromatic clusters they originate from (Schneider et al., 2010). B3CA and B4CA can be produced from small condensed units of 3 aromatic rings minimum (e.g. retene), while formation of B6CA requires a minimum of five or more condensed rings (Ziolkowski et al., in press). In the soluble and colloidal fractions of the aged char (Fig. 1), the larger content in B6CA indicates that larger molecular clusters became soluble due to oxidation with ageing (Dittmar and Koch, 2006), while such larger clusters are absent in the corresponding fractions from fresh chars. Thus, with residence time in soil, larger and more chemically condensed clusters were released.

The BPCA patterns of the aged char soluble fraction (Fig. 1) are similar to patterns found in dissolved organic matter of Apalachee river bay in Dittmar (2008) or Suwannee river in Ziolkowski and Druffel (2010), i.e. B4CA and B5CA represent the majority of the distribution, while B3CA and B6CA represent 15 and 5% of the total, respectively. If this observation would hold true for PyC breakdown patterns, this would indicate that no major chemical modification happens to the colloidal and dissolved PyC, between ageing in soil and on their way to the river.

The soluble and colloidal fractions extractable from freshly pyrolysed char material are small. With residence time in soil, char releases larger soluble pyrogenic condensed aromatic structures. This soluble and colloidal PyC has a molecular marker pattern similar to soluble PyC found in river DOM.

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Manuscript 4

Charcoal quality does not change over a century in a tropical agro-ecosystem

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Short Communication

Charcoal quality does not change over a century in a tropical agro-ecosystem

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ABSTRACT

Charcoal stocks were determined in a chronosequence of soils which have been converted to agricultural land use by slash-and-burn up to 100 years ago. With time, opposite to our assumptions, the charcoal chemical quality, as measured by molecular markers for pyrogenic carbon, did not change and charcoal stocks did not show a clear decrease. Our results indicate that charcoal may resist chemical degradation even when exposed to intense weathering in a tropical climate.

1. Introduction

Charcoal, also referred to as fire-derived or pyrogenic carbon (PyC), is a residue of incomplete combustion of biomass and is ubiquitous in soils, but loss processes and rates are still poorly understood (Preston and Schmidt, 2006). Up to now there are only a few studies investigating the long-term (i.e. decadal) fate of PyC in soils, and results are ambiguous. Short-term (up to 3.2 years) laboratory incubation experiments revealed that <2% of PyC produced at ≥200 °C was mineralized (Baldock and Smernik, 2002; Hamer et al., 2004; Bruun et al., 2008; Kuzyakov et al., 2009; Nguyen et al., 2010; Zimmerman, 2010). There are only two published long-term (100 years) field studies which observed loss of PyC in tropical soil by spectroscopy (Nguyen et al., 2008: 70% loss), or in a steppe soil by molecular marker measurements (Hammes et al., 2008: 25% loss), respectively. Nguyen et al. (2008) found increasing oxidation at the surface of manually isolated PyC pieces using X-ray photoelectron spectroscopy (XPS) in the tropical soil. However, qualitative changes of the finely distributed PyC in the bulk soil have not been investigated so far. The unique advantage of the molecular marker method over the previously used spectroscopic methods is to gain greater insight into changes in chemical quality over time of PyC in the bulk material. For example, one of the molecular markers, B6CA, is particularly useful to estimate the degree of aromatic condensation in the samples (Hammes et al., 2008; Schneider et al., 2010). Indeed, qualitative changes in the bulk material, i.e. preferential accumulation of more condensed aromatic backbone of the PyC structures, were found for the steppe soils using the molecular marker method (Hammes et al., 2008). Here we applied the molecular marker method used in the steppe soil (Hammes et al., 2008) to the tropical soil samples (Nguyen et al., 2008) to follow the PyC in a soil chronosequence (2, 3, 5, 20, 30, 45, 80, 100 years since the last PyC deposition). We hypothesized that we would find a selective enrichment of more condensed (and thus more chemically stable) forms of PyC in the bulk soil.

2. Materials and methods

Following a space-for-time approach, the soil samples (Humic Nitosol, FAO-UNESCO, 1998) were collected in an area where forests were converted from forest to agricultural land by slash-and-burn practice up to 100 years ago. Soils were under permanent cultivation with no new fires since the conversion. The area is located in South Nandi (00°04′30″ N, 34°58′34″ E), western Kenya with altitudes ranging from 1600 to 1800 m above sea level, mean annual temperature of about 19 °C and mean annual precipitation of about 2000 mm (Nguyen et al., 2008). Nine 200 cm^3 core subsamples from the upper 0.1 m of soil were sampled in each field

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and then combined into one composite soil sample (Solomon et al., 2007; Kimetu et al., 2008; Kinyangi, 2008; Nguyen et al., 2008). This chronosequence opened the unique possibility to investigate changes in bulk PyC over 100 years in a space-for-time approach. As a trade-off we had to accept the associated uncertainties, including the varying (and to us unknown) amounts of PyC produced during the different fires, and possible deposition of PyC from near-by burns.

In this study we used benzene polycarboxylic acids (BPCA) molecular markers for pyrogenic carbon (PyC) assessment (Glaser et al., 1998; Brodowski et al., 2005; Schneider et al., 2010). Briefly, samples ($n = 3$) were pretreated with 4M trifluoro acetic acid (4 h, 105 °C), followed by conversion of PyC into BPCA by nitric acid oxidation (8 h, 170 °C). The digest was purified and subsequently derivatized and analyzed on a gas chromatograph equipped with flame ionization detector. The acids with 3, 4, 5, and 6 carboxyl functions (B3CA, B4CA, B5CA, and B6CA, respectively) were identified and summed up to represent the amount of pyrogenic molecular markers derived from the material. Data was normalized to the BPCA-C content measured in a reference soil (Table S1) (Schneider et al., 2010).

3. Results and discussion

3.1. Quality of PyC

The most striking feature of our data is that the proportions of B6CA remained constant over a century at about 35% (Fig. 1a), which is in contrast to the previous observation of Hammes et al. (2008) for a steppe soil. There, decreasing PyC stocks (−25%) over a century had been accompanied by a preferential accumulation of the highly condensed, aromatic backbone of PyC (Hammes et al., 2008), indicated by increasing proportions of B6CA.

It seems that chemical changes, such as increasing oxidation, are limited to the surface of PyC particles, whereas the more protected PyC particle inside remained largely unaffected by oxidation processes (Nguyen et al., 2008). There are indications that coarser PyC particles after deposition are initially degraded to finer particles (Bird et al., 1999) and then subsequently protected from further degradation by interaction with soil minerals (Nguyen et al., 2008), which could explain the absence of chemical changes in the finely distributed bulk PyC, which was investigated here.

3.2. Quantity of PyC

Both organic carbon (OC) and PyC had been present in the forest before land conversion (labeled as “forest” in Fig. 1), and the last PyC input happened when land was converted to agriculture using slash-and-burn (Nguyen et al., 2008). After conversion and with increasing time of agricultural use, OC stocks decreased rapidly (Fig. 1b), an observation typically made for such land use changes (e.g. Brady and Weil, 2001). For PyC stocks quantified by BPCA, no clear trends could be observed. If there were trends with time, they were obscured by the large spatial heterogeneity of PyC stocks. To test our results for plausibility, we compared them to earlier results by Nguyen et al. (2008), who measured PyC stocks on identical samples but with a different method (nuclear magnetic resonance spectroscopy combined with a molecular mixing model, NMR-MMM). As a result, data of both methods correlate well ($r^2 = 0.80$, insert Fig. 1b), and show the typical systematic offset, with BPCA values being approximately 1/5 of those measured by NMR-MMM. The systematic offset reflects the fundamentally different principles of the two methods. The BPCA method measures molecular markers released upon wet chemical oxidation as a representative subfraction of PyC, while NMR-MMM measures the contribution of aryl C to the NMR spectrum and from that calculates the content of PyC (Nelson and Baldock, 2005; Hammes et al., 2007; Kaal et al., 2008).

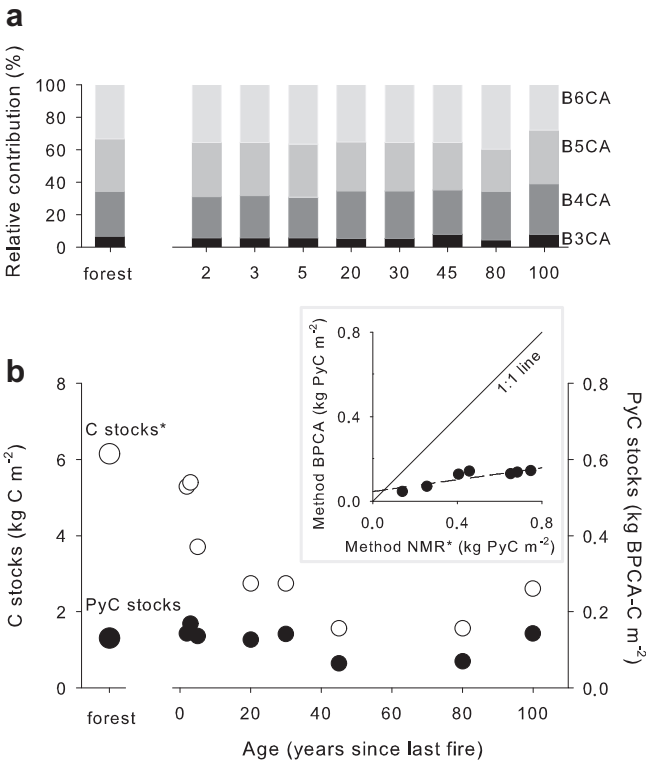

Fig. 1. The PyC quality (a) and quantity of bulk soil organic carbon (SOC) and the fire-derived PyC subfraction (b) in soil chronosequence samples with increasing time of conversion from forest to agricultural land by slash-and-burn. The “forest” sample represented the pre-existing OC and PyC stocks in the forest soil. a) Relative contributions of BPCA marker molecules (B3CA: hemimellitic and trimellitic acid; B4CA: prehnitic, mellophanic and pyromellitic acid, B5CA: pentacarboxylic acid; B6CA: mellitic acid). B6CA, a measure for the degree of condensation in PyC, did not change over the observation period. ($n = 3$; 45 years sample $n = 1$) b) SOC (white circles, left scale) and PyC stocks, measured as benzene polycarboxylic acids carbon (BPCA-C) (black circles, right scale). Insert: comparison of PyC stocks measured by nuclear magnetic resonance spectroscopy with molecular mixing model (NMR-MMM; Nelson and Baldock, 2005) (Method NMR, *x*-axis) and BPCA molecular markers (Method BPCA, *y*-axis). Method BPCA yielded consistently lower numbers, but both methods showed a close linear relationship (dashed line, $r^2 = 0.80$, $y = 0.045 + 0.14$*x, $p = 0.05$). Standard errors for analytical replicates ($n = 3$) are smaller than symbol size. *Organic carbon stocks and NMR-MMM data taken from Nguyen et al. (2008).

4. Conclusions

Over a century of weathering in a tropical climate,

- The space-for-time approach used in this study showed that OC stocks clearly decreased, but total PyC stocks did not.
- We found no indications for a changing chemical quality of the bulk PyC, although we expected the decomposition of less stable PyC fractions to be accompanied by a relative enrichment of highly condensed aromatic PyC fractions.

Interestingly, we applied the molecular marker method (BPCA) to bulk soil samples and our data on quantity and quality of PyC is consistent with those results obtained by NMR-spectroscopy for quantification of PyC and X-ray photoelectron spectroscopy for chemical properties of hand-picked ground and un-ground char particles.

Author contributions

The study was proposed jointly by all authors. JL provided sample material, additional data and information about the sampling sites. The experiments and data analysis were carried out by MPWS. Paper writing was completed by MPWS under supervision of MWIS, with contributions from JL.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2011.05.020.

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Supplementary Material

Table S1: BPCA-C concentrations measured in the studied soils, compared to a reference soil (called Chernozem in Hammes et al., 2007). We co-run this reference soil with each sample set and use the results to normalize the BPCA data (SE, n=3). BPCA data is calculated without the correction factor 2.27 proposed by Glaser et al. (1998).

sample (years since BC deposition)	measured BPCA	normalized BPCA	Chernozem BPCA	factor
	---------- g C kg^{-1} soil ----------			
forest	1.82±0.14	1.94±0.15	1.05±2.10	1.06
2	2.62±0.10	2.03±0.07	1.44±0.36	0.77
3	2.72±0.10	2.11±0.08	1.44±0.36	0.77
5	2.20±0.02	1.71±0.01	1.44±0.36	0.77
20	0.97±0.01	1.27±0.02	0.86±0.79	1.30
30	1.02±0.07	1.33±0.09	0.86±0.79	1.30
45	0.38	0.40	1.05±2.10	1.06
80	0.47±0.02	0.62±0.02	0.86±0.79	1.30
100	1.20±0.03	1.28±0.03	1.05±2.10	1.06
		mean:	1.12	1.00

PART C – Appendix

Structure formulae of benzene polycarboxylic acids

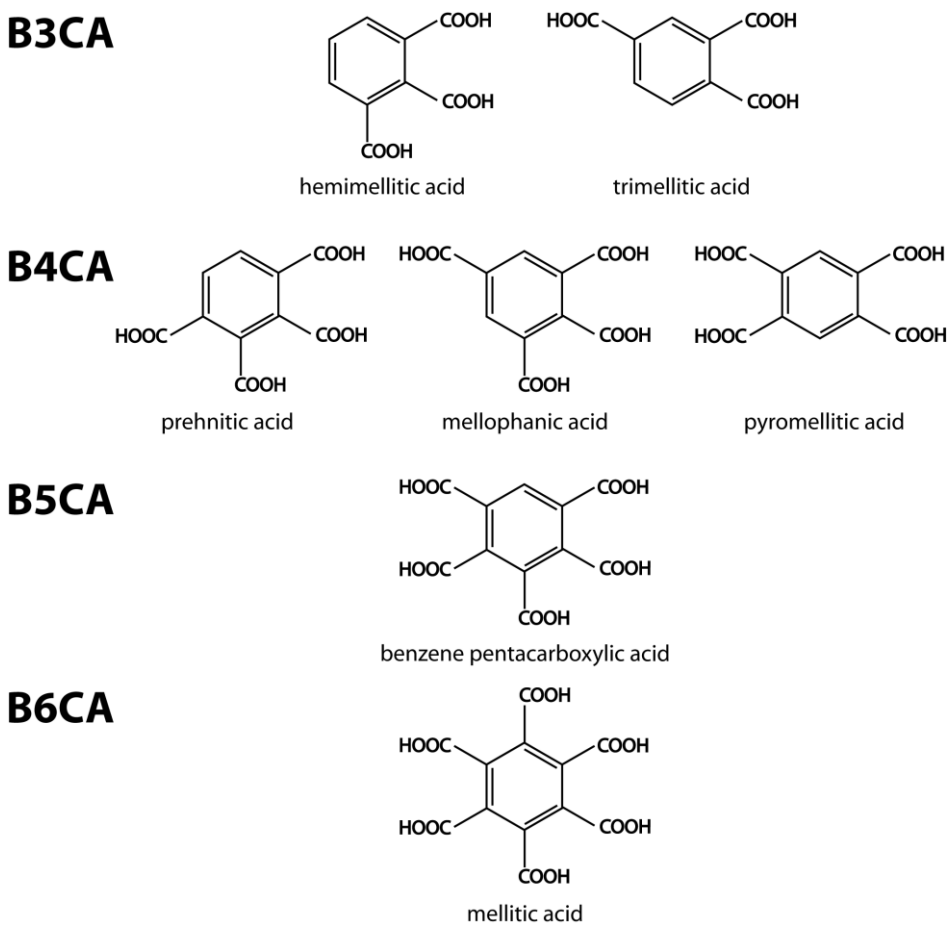

Figure A1: Structures of benzene polycarboxylic acids used as markers for pyrogenic carbon (PyC)

Protocol for analysis of benzene polycarboxylic acids analysis using GC

Before starting with sample preparation, the conditioning of the cation exchange resin (DOWEX 50W X 8-400, Sigma-Aldrich, Steinheim, Germany) and the conversion to H^+ form should be done:

a. Rinse with 2 column volumes deionized water
b. Rinse with 1 column volume 2 M NaOH
c. Rinse with 3 column volumes deionized water (check if pH-value of elute is neutral)
d. Rinse with 1 column volume 2 M HCl
e. Rinse with 2 column volumes deionized water (check conductivity < 2 µS)

Step 1: Sample preparation before HNO_3 digestion

Weigh in ca. 400-500 mg soil or 2-100 mg of charcoals in quartz digestion tubes. All analyses are done in triplicate. Include two replicates of a reference material (e.g. Chernozem soil, Hammes et al., 2007) and one blank sample. Add 10 mL 4 M trifluoroacetic acid (TFA) and digest samples in a pre-heated oven at 105 °C for 4 hours. Let it cool down for 1 hour at room temperature. Filter over quartz filter (Whatman GF6, Schleicher & Schuell, Dassel, Germany), wash with excess of water and dry filters for two hours at 40 °C.

Step 2: Conversion to BPCA

Scrape the sample carefully off the filter paper into the digestion tubes. For small sample amounts (charcoals), put the whole filter with sample into the tube. Add 2 mL 65% (m/m) HNO_3 and digest samples at 170 °C for 8 hours in a preheated oven.

Step 3: Sample preparation after HNO_3 digestion

Filter samples through an ashless cellulose filter (Whatman 589/3, Schleicher & Schuell, Dassel, Germany) into volumetric flasks and fill up to 10 mL.

Step 4: Removal of cations

a. Place 100 µL of the internal standard in H_2O on the cation exchange resin column
b. Add 2 mL aliquot of the BPCA extract on the column
c. Wash the column 5 times with 10 mL deionized water. The water has to be completely washed through before adding the next 10 mL.
d. Freeze the flasks with liquid nitrogen and freeze dry overnight on the freeze drier

Step 5: Derivatization

a. Transfer the samples with 4 times 1 mL methanol into a GC vial and dry at 45 °C for about 40 min in a concentrator (Concentrator plus, Eppendorf, Hamburg, Germany). Check for complete dryness.
b. Pipette 100 µL N,O-bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA+TMCS 99:1, Supelco, Bellefonte PA, United States) and 100 µL pyridine into the vials
c. Close the vials with white plastic caps and septum and derivatize the samples at 80 °C for 2 hours on a pre-heated aluminum block
d. Let the samples stand for at least 24 hours before injection on the GC
e. Transfer the samples to inlets in GC vials (split into two) and close tightly with plastic cap and septum

Step 6: Chromatographic analysis

Gas chromatographic analysis was performed on an Agilent 6890 gas chromatograph, equipped with a flame ionization detector (FID) and a DB-5MS capillary column (50 m x 0.2 mm i.d., 0.33 µm film thickness). Helium was used as a carrier gas at a constant flow of 0.8 mL min^{-1}. Both the injector and detector temperatures were 300 °C. Aliquots (2 µL) of sample solution were injected at a split ratio of 30:1 into a fully deactivated inlet system with silylated liners. The temperature program was: initial column temperature of 100 °C held for two minutes, followed by an increase of 20 °C min^{-1} to 240 °C and held for seven minutes. Subsequently, the temperature was raised by 30 °C min^{-1} to 300 °C and held for additional 15 minutes.

Standard series

A standard series was prepared for each analysis run using standard solutions of 20, 40, 60, 80, 100 and 120 µg BPCA per vial. The solution was prepared by dissolving 100 µg of each BPCA in 100 µL methanol and transferring the corresponding volume of standard solution into the vial. The internal standard, phthalic acid, was added as 100 µg in 100 µL methanol. Subsequently, the standards were completely dried and individually derivatized as described above.

Protocol for analysis of benzene polycarboxylic acids analysis using HPLC

Step 1: Sample preparation before HNO_3 digestion

Weigh in ca. 2-10 mg of charcoals in quartz digestion tubes. All analyses are done in triplicate.

Step 2: Conversion to BPCA

Add 2 mL 65% (m/m) HNO_3 and digest samples at 170 °C for 8 hours in a preheated oven.

Step 3: Sample preparation after HNO_3 digestion

Preparation of solvents as follows:

Solvent A:

ortho phosphoric acid [50%] 1.0 mL L^{-1}

Tetrabutylammonium bromide (TBAB) 2 g L^{-1}

- dissolved in ultrapure water

- adjust to pH 8 by slowly adding 1 M NaOH

Solvent B:

Solvent A + 75 vol.-% methanol

Dry 1 mL aliquot of the samples at 60 °C under N_2 stream to complete dryness. Redissolve in 1 mL mixture of methanol and water (25:75 v:v). Aliquots of 20-100 µL were transferred to 1 mL HPLC vials and filled up to 1 mL with Solvent A. Check pH for each sample (must not be below pH 4).

Step 4: Removal of cations

-

Step 5: Derivatization

-

Step 6: Chromatographic analysis

High Performance Liquid Chromatography (HPLC) analysis was carried out on a Thermo Surveyor HPLC instrument equipped with a reversed phase Waters Atlantis T3 column (150 mm x 2.1 mm, 3 µm) applying a gradient of phosphate buffer (pH 8) modified with tetrabutylammonium bromide (TBAB) (2 g L^{-1}) and methanol over 48 minutes (Table A1). The injection volume was 20 µL. A photo diode array detector (DAD) was used for peak identification (absorbance spectra 220-380 nm) and quantification (absorption at 240 nm).

Table A1: Mobile phase mixing gradients for HPLC.

Time (min)	Mobile phase B (vol.-%)
0.01	Start
0.02	40
48	47
49	100
53	100
54	40
64	40
64.01	Stop

Standard series

A standard series was prepared for using standard solutions of 2, 4, 6 and 8 µg BPCA per vial. The solution was prepared by dissolving 100 µg of each BPCA in 100 µL mixture of methanol and water (25:75 v:v) and transferring the corresponding volume of standard solution into the vial, which was subsequently filled up to 1 mL with Solvent A.

Data supplements to Manuscripts 1, 2 and 4

Table A2: Remaining mass after pyrolysis and elemental composition of charcoals produced from wood and grass at different temperatures (Manuscript 1 and 2).

Sample	mass remaining [%]	C [%]	H [%]	N [%]	O [%]	H/C atomic ratio	O/C atomic ratio
wood 200°C	88.0	50.3	5.6	0.1	44.2	1.32	0.66
wood 250°C	71.0	54.3	5.3	0.1	40.1	1.16	0.55
wood 275°C	52.5	64.1	4.3	0.2	31.4	0.79	0.37
wood 300°C	44.8	69.5	4.0	0.2	26.1	0.69	0.28
wood 350°C	41.0	73.4	3.2	0.3	23.0	0.52	0.24
wood 400°C	31.0	78.1	3.0	0.3	18.5	0.46	0.18
wood 500°C	30.7	87.1	2.7	0.3	9.8	0.36	0.08
wood 600°C	25.5	93.8	1.9	0.3	3.9	0.24	0.03
wood 700°C	27.5	95.1	1.1	0.5	3.3	0.14	0.03
wood 800°C	27.0	96.0	0.7	0.7	2.4	0.08	0.02
wood 900°C	25.0	96.5	0.3	0.8	2.2	0.04	0.02
wood 1000°C	27.0	96.3	0.2	1.0	2.5	0.03	0.02
grass 200°C	86.0	46.2	5.4	0.8	39.2	1.38	0.64
grass 250°C	62.8	52.1	5.2	0.9	32.5	1.18	0.47
grass 275°C	49.0	58.5	4.6	1.4	24.3	0.94	0.31
grass 300°C	45.3	59.2	4.3	1.1	22.7	0.86	0.29
grass 350°C	39.4	62.1	3.6	1.2	20.0	0.70	0.24
grass 400°C	35.0	63.1	3.0	1.0	16.2	0.57	0.19
grass 500°C	32.2	66.8	2.3	0.9	11.3	0.40	0.13
grass 600°C	33.0	67.4	1.5	0.8	9.6	0.27	0.11
grass 700°C	27.9	69.8	1.1	0.8	11.6	0.18	0.12
grass 800°C	27.1	66.6	0.8	1.1	9.8	0.14	0.11
grass 900°C	23.8	71.0	0.5	0.9	9.1	0.08	0.10
grass 1000°C	21.9	72.1	0.3	0.7	7.0	0.05	0.07

Table A3: BPCA yields and relative distributions from wood charcoal thermosequence samples, data from GC-BPCA and LC-BPCA measurements (Manuscript 1 and 2)

Sample	BPCA-C [g kg^{-1} OC]		B3CA [%]		B4CA [%]		B5CA [%]		B6CA [%]	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Method: GC-BPCA										
wood 200 °C	3.3	1.2	30.4	15.2	54.1	9.3	9.9	5.8	5.6	5.6
wood 250 °C	18.3	0.7	16.7	1.2	57.6	2.6	17.9	3.7	7.7	0.2
wood 275 °C	68.6	2.0	4.6	0.0	28.1	0.0	34.4	0.3	32.9	0.3
wood 300 °C	83.2	10.8	8.0	0.9	31.8	2.4	35.1	3.2	25.2	0.2
wood 350 °C	94.1	2.3	5.8	0.3	27.6	0.9	30.7	1.0	35.9	0.3
wood 400 °C	127.8	5.2	6.0	0.2	25.1	0.5	33.9	0.6	35.0	0.6
wood 500 °C	140.1	3.4	7.6	0.3	24.2	0.5	29.6	0.4	38.6	0.6
wood 600 °C	136.6	5.1	4.0	0.1	14.9	0.1	25.1	0.2	56.1	0.3
wood 700 °C	155.3	8.0	2.2	0.0	8.5	0.3	18.5	0.2	70.8	0.5
wood 800 °C	103.7	8.2	0.0	0.0	4.6	0.4	17.3	0.9	78.1	1.2
wood 900 °C	83.4	2.0	0.0	0.0	0.0	0.0	14.8	1.6	85.2	1.6
wood 1000 °C	85.7	15.3	0.0	0.0	0.0	0.0	2.3	2.3	97.7	2.3
Method: LC-BPCA										
wood 200 °C	6.0	0.0	28.1	0.2	37.9	0.1	18.7	0.1	15.3	0.1
wood 250 °C	33.3	0.3	7.3	0.1	35.1	0.1	32.0	0.1	25.5	0.1
wood 275 °C	85.1	0.6	8.2	0.0	32.1	0.0	34.0	0.0	25.8	0.1
wood 300 °C	115.1	0.8	7.0	0.2	32.2	0.2	34.2	0.0	26.6	0.1
wood 350 °C	163.1	1.2	7.6	0.4	31.2	0.2	33.1	0.1	28.1	0.1
wood 400 °C	198.7	6.6	7.9	0.2	32.2	0.1	31.8	0.1	28.0	0.1
wood 500 °C	208.9	6.7	6.8	0.1	34.7	0.1	27.8	0.0	30.7	0.1
wood 600 °C	240.2	1.8	5.0	0.2	23.6	0.2	24.6	0.1	46.9	0.1
wood 700 °C	232.3	5.2	4.9	0.1	12.9	0.1	15.8	0.0	66.4	0.2
wood 800 °C	233.9	7.8	6.1	0.2	14.2	0.4	9.6	0.1	70.0	0.7
wood 900 °C	213.4	2.1	0.0	0.0	15.6	0.4	8.8	0.2	75.6	0.6
wood 1000 °C	197.7	9.0	0.0	0.0	14.5	0.7	7.7	0.3	77.8	1.0

Table A4: BPCA yields and relative distributions from grass charcoal thermosequence samples, data from GC-BPCA and LC-BPCA measurements (Manuscript 2)

Sample	BPCA-C [g kg^{-1} OC]		B3CA [%]		B4CA [%]		B5CA [%]		B6CA [%]	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Method: GC-BPCA										
grass 200 °C	7.22	0.48	13.76	2.26	44.39	2.54	37.49	2.99	4.36	4.36
grass 250 °C	31.67	2.65	8.00	0.27	34.69	1.12	34.74	0.25	22.57	1.54
grass 275 °C	59.48	1.15	8.16	0.03	29.18	0.35	37.74	0.29	24.93	0.22
grass 300 °C	64.81	9.15	10.86	1.04	32.80	2.21	30.62	1.50	25.73	1.75
grass 350 °C	100.40	1.73	7.28	0.07	29.21	0.17	35.92	0.18	27.59	0.05
grass 400 °C	109.27	1.38	7.98	0.04	30.45	0.29	30.76	0.22	30.80	0.50
grass 500 °C	128.24	3.61	4.13	0.03	18.86	0.12	30.30	0.13	46.71	0.21
grass 600 °C	142.79	7.62	2.78	0.19	12.86	0.88	26.04	0.67	58.32	1.73
grass 700 °C	125.71	2.79	1.72	0.02	8.30	0.04	17.34	0.29	72.64	0.32
grass 800 °C	119.79	8.14	0.00	0.00	3.91	0.21	12.83	0.54	83.26	0.75
grass 900 °C	121.79	3.77	0.00	0.00	1.26	0.63	12.00	0.26	86.74	0.56
grass 1000 °C	114.29	5.17	0.00	0.00	0.53	0.29	7.25	0.41	92.22	0.49
Method: LC-BPCA										
grass 200 °C	8.10	0.17	23.14	0.22	37.75	0.04	25.92	0.21	13.19	0.07
grass 250 °C	45.38	0.80	7.00	0.14	38.40	0.09	33.51	0.04	21.08	0.04
grass 275 °C	79.83	0.38	5.53	0.06	38.57	0.10	33.29	0.07	22.61	0.07
grass 300 °C	107.31	1.36	4.72	0.04	38.67	0.12	32.91	0.07	23.70	0.04
grass 350 °C	146.82	2.32	4.24	0.07	39.80	0.10	31.58	0.04	24.39	0.00
grass 400 °C	178.09	1.89	3.68	0.04	42.61	0.08	28.79	0.05	24.92	0.02
grass 500 °C	204.69	1.32	3.97	0.11	35.25	0.07	25.34	0.04	35.45	0.04
grass 600 °C	212.28	0.75	2.04	0.03	19.32	0.03	21.63	0.10	57.01	0.15
grass 700 °C	197.25	4.22	1.82	0.07	12.49	0.12	14.52	0.35	71.17	0.39
grass 800 °C	188.28	3.64	2.75	0.15	9.69	0.30	8.46	0.45	79.10	0.49
grass 900 °C	172.50	1.72	2.71	0.13	8.61	0.22	6.13	0.14	82.55	0.48
grass 1000 °C	158.25	5.30	0.00	0.00	7.87	0.33	4.19	0.05	87.95	0.37

Table A5: BPCA yields and relative distributions from reference materials, 450 °C wood charcoal and 450 °C grass charcoal (standard error (SE), n=3)

Sample	BPCA-C [g kg^{-1} OC]		B3CA [%]		B4CA [%]		B5CA [%]		B6CA [%]	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Method: GC-BPCA										
wood 450 °C	137.22	2.92	6.00	0.08	26.18	0.33	36.10	0.42	31.72	0.05
grass 450 °C	116.40	7.50	5.88	0.11	26.36	0.22	36.26	0.18	31.50	0.14
Method: LC-BPCA										
wood 450 °C	192.55	1.70	2.53	0.01	34.54	0.13	34.00	0.03	28.94	0.17
grass 450 °C	165.26	0.44	4.14	0.06	41.78	0.09	30.24	0.00	23.84	0.14

Table A6: BPCA yields and relative distributions from reference material, Chernozem soil, from three independent repeated analyses, data taken from Manuscript 4 (standard error (SE), n=3).

Sample	BPCA-C [g kg^{-1} OC]		B3CA [%]		B4CA [%]		B5CA [%]		B6CA [%]	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Method: GC-BPCA										
Chernozem (1)	52.19	2.10	4.70	0.58	23.50	0.69	30.82	1.08	40.98	0.56
Chernozem (2, n=2)	42.72	0.79	3.41	0.12	25.21	0.60	28.12	0.39	43.26	1.11
Chernozem (3, n=2)	71.71	0.36	3.43	0.05	19.85	0.19	32.12	0.02	44.60	0.22

Table A7: BPCA yields and relative distributions from soil chronosequence samples (from Manuscript 4) and basic soil properties (data taken from Nguyen et al., 2009)

Sample	BPCA-C [g kg^{-1} OC]		B3CA [%]		B4CA [%]		B5CA [%]		B6CA [%]		Carbon [%]	Nitrogen [%]	C/N	Bulk density [Mg m^{-3}]
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE				
Method: GC-BPCA														
forest	19.87	1.49	6.55	0.44	27.83	1.47	32.36	0.30	33.26	1.63	9.18	0.95	9.96	0.67
2 years	34.71	1.27	5.78	0.08	25.31	0.17	33.22	0.16	35.68	0.27	7.56	0.73	10.32	0.70
3 years	40.41	1.54	5.90	0.09	25.88	0.09	32.71	0.36	35.51	0.23	6.74	0.72	9.30	0.80
5 years	47.69	0.39	5.80	0.06	24.92	0.31	32.58	0.06	36.70	0.41	4.62	0.49	9.42	0.80
20 years	35.57	0.51	5.43	0.18	29.29	0.58	30.16	0.94	35.11	0.20	2.74	0.29	9.53	1.00
30 years	39.60	2.59	5.26	0.16	29.46	0.76	29.72	1.27	35.56	0.35	2.58	0.29	9.00	1.06
45 years	38.99	-	7.96	-	27.42	-	29.07	-	35.55	-	1.39	0.15	9.18	1.12
80 years	34.17	1.19	4.35	0.10	29.91	0.24	25.98	0.26	39.76	0.49	1.39	0.15	9.27	1.12
100 years	51.85	1.08	7.95	0.15	31.08	0.46	32.92	0.73	28.05	1.28	2.32	0.24	9.85	1.12

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Curriculum vitae

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Education

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09/2003 – 07/2004	Universidad de León (Spain), Environmental science (Ciencias ambientales)
10/1999 – 09/2001	Vordiplom in Geoecology (Undergraduate studies), Technical University Carolo-Wilhelmina at Braunschweig (Germany)
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Academic positions & Research experience

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Contributions at international conferences

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Publication of Diplom thesis

Schneider M.P.W., Scheel T., Mikutta R., van Hees P., Kaiser K. und Kalbitz, K. (2010): Sorptive stabilization of organic matter by amorphous Al hydroxide, Geochimica et Cosmochimica Acta 74, 1606-1619.

… aber er meine,
es müsse
ein unendliches
Wonnegefühl sein,
so von dem
eigentümlichen Leben
jeder Form
berührt zu werden,
für Gesteine, Metalle,
Wasser und Pflanzen
eine Seele zu haben,
so traumartig
jedes Wesen
in der Natur
in sich aufzunehmen,
wie die Blumen
mit dem Zu-
und Abnehmen
des Mondes die Luft …

Georg Büchner, Lenz